JIMMAUNIVERSITY
COLLEGEOFAGRICULTUREANDVETERINARYMEDICINE
SCHOOLOFVETERINARYMEDICINE

BOVINEBRUCELLOSIS;SEROPREVALENCE, ASSOCIATED RISK FACTORS ANDASSESSMENT OFKNOWLEDGE, ATTITUDEANDPRACTICEOFFARMOWNERSINSELECTEDDISTRICTSOFEA STWOLLEGAZONE,OROMIA,ETHIOPIA

BY

WAKUMAMITIKU

NOVEMBER2019

JIMMA,ETHIOPIA

JIMMAUNIVERSITY
COLLEGEOFAGRICULTUREANDVETERINARYMEDICINE
SCHOOLOFVETERINARYMEDICINE

BOVINE BRUCELLOSIS; SEROPREVALENCE, ASSOCIATED RISK FACTORS ANDASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICE OF FARM OWNERS IN SELECTED DISTRICTS OF EAST WOLLEGA ZONE, OROMIA, ETHIOPIA

AThesisSubmittedtotheSchoolofVeterinaryMedicine,JimmaUniversityCollegeofAgricultureandVeterinaryMedicineinPartialFulfillmentoftheRequirementsfortheDegreeof MasterofScienceinVeterinaryPublicHealth

BY

WAKUMAMITIKU

MAJORADVISOR:Dr.MOTUMADEBELO(DVM,MSc.AssistantProfessor)

CO-ADVISOR:Prof.TADELETOLOSA(DVM,MSc,PhD)

November2019
Jimma,Ethiopia

SCHOOLOFGRADUATESTUDIES

JIMMAUNIVERSITY

COLLEGEOFAGRICULTUREANDVETRINARYMEDICINE

MSc THESIS APPROVAL SHEET

We,theundersigned,memberoftheboardexaminersofthefinalopendefensebyWakumaMitiku havereadandevaluatedhis/herthesisentitled"**BovineBrucellosis;Seroprevalence,Associatedriskfactors and Assessment of Knowledge-Attitude and Practice of Farm Owners on BrucellosisinSelectedDistrictsofEastWollegaZone,Oromia,Ethiopia"**andexaminedthecandidate.Thisisthereforetocertifythatthethesishasacceptedinpartialfulfillmentsforthedegree MasterofSciencein**VeterinaryPublicHealth.**

_____/____/_____
NameofChairpersonSignatureDate

Dr Motuma Debelo_____/____/_____
NameofMajorAdvisorSignatureDate

Dr.MekonnenAddis_____/____/_____
NameofInternalExaminerSignatureDate

_____ _____ ____/___/____
NameofExternalExaminersignatureDate

DEDICATION

Iwishtodedicatethisthesistomyfather**MitikuGemechu**andmymother**NechoAbdeta**,whogroundedafirmfoundationofmyacademicjourney.Theircontributionisenormousindeed,thatIcannotquantify.Idedicateitalsotomybelovedwife**BiftuFentahun**forbeingpatient,providingmoralsupport,encouragementandinspirationfortwoyearsstayattheUniversity.

STATEMENTOFTHEAUTHOR

First,Ideclarethatthisthesisismyoriginalworkandthatallsourcesofmaterialusedforthisthesisha sbeendulyacknowledged.Thisthesishasbeensubmittedinpartialfulfillmentoftherequirements forapostgraduate(MSc)degreeatJimmaUniversity,CollegeofAgricultureandVeterinaryMedi cineandisdepositedattheUniversity/Collegelibrarytobemadeavailabletoborrowersunderrules oftheLibrary.Isolemnlydeclarethatthisthesisisnotsubmittedtoanyotherinstitutionanywherefo rtheawardofanydegree.Briefquotationsfromthisthesisareallowablewithoutspecialpermission providedthataccurateacknowledgementofsourceismade.Requestsforpermissionforextended quotationfromorreproductionofthismanuscriptinwholeorinpartmaybegrantedbytheheadofth emajorschoolorthedeanoftheschoolofgraduatestudieswheninhisorherjudgmenttheproposed useofthematerialisintheinterestsofscholarship.Inallotherinstances,howeverpermissionmustb eobtainedfromtheauthor.

Name:WakumaMitikuGemechuSignature:_____

Place:JimmaUniversityCollegeofAgricultureandVeterinaryMedicine

DateofSubmission:_____

BIOGRAPHICALSKETCH

WakumawasbornonApril4,1986G.CfromhisfatherMitikuGemechuandhismotherNechoAbdetainIlamuMelolekebele,HababoGudurudistrict,HoroGuduruWollegazone,Oromiaregion,Ethiopia.Heattendedhisprimaryeducation(1-6)inKenateBiyaelementaryschoolandjunioreducation(7-8)inImbabojuniorandelementaryschoolstartingfrom1993years.Heattendedhissecondaryeducation(9-10)inFinchaseniorsecondaryschoolandhealsoattendedhispreparatoryschoolinShambupreparatoryschooluntil2004.AfterhecompletedhispreparatoryschoolhejoinedJimmaUniversityCollegeofAgricultureandVeterinaryMedicinein2005andhewasawardedwithDoctorofVeterinaryMedicine(DVM)degreeinVeterinaryMedicineonJune,2009.Aftergraduation,hewasemployedasInstructorofAnimalHealthcoursesinHoroGuduruCollegeFinchaCampusfromJanuary2010toAugust2012forconsecutivethreeyears.ThenafterhewasemployedasVeterinarianinJimmaGenetiWoredaLivestockandFisheryResourceofficeasworkprocesscoordinatorfromFebruary,2013-2017ortillhejoinedJimmaUniversityCollegeofAgricultureandVeterinaryMedicinetopursueMasterofScience(MSc)degreeinVeterinaryPublicHealth.

ACKNOWLEDGEMENTS

Aboveall,thankstomyalmightyGodforhelpingivingmecouragetocopupcomplicatedsituation sIfacedforpursuingmystudyandcourageduringmywholestudytime.

Next,Iwouldliketoexpressmydeepestandsinceregratitudetomyacademicadvisors Dr.Motuma DebeloandProf.TadeleTolosafortheirunreservedprofessionaladviceinimplementingtheresea rchdesign,kindcooperationandconstructivecommentsandsuggestionsincorrectingthisthesist oproducefinalversion.

IwanttoexpressmydeepestgratitudeandappreciationtoBedeleRegionalVeterinaryLaboratory anditsstaffmembersespeciallytoDr.FikaduBekeleforprovidingmelaboratoryfacilities,cooper ationduringthefieldresearchworks,andtechnicalassistanceduringsampleprocessingandanaly sis.

Again,IwanttoexpressmydeepestgratitudeandappreciationtoGobuSeyo,SibuSireandGudeya Biladistricts-livestockandfisheryoffice,East-Wollegazone,Ethiopia,especiallytoDr.SisayBekeleandAtoDessalegnMerdasafortheirpositiv ecooperationandfieldguidanceduringsamplecollectionfromtheDistricts.

Finally,myunreserved,heartfeltgratitudealsogoestomywife,w/roBiftuFentahunforherunreser vedencouragementandtolerancewhilethisdocumentwaspreparedandtomyfamiliesfortheirmo ralsupportthroughmyentireeducationalcareer.

TABLEOFCONTENTSPAGES

LISTOFTABLESPAGES

LISTOFFIGURESPAGES

LISTOFANNEXESPAGES

LISTOFABBREVASIONSANDACRONYMS

AASUR AddisAbabaandSurroundings

AI ArtificialInsemination

ARNS AmharaRegionalNationalState

B.abortus *Brucellaabortus*

bvsbiovars

C-ELISACompititiveEnzymeLinkedImmunoSorbentAssey

CFT ComplementFixationTest

CFUColonyFormingUnit

CHE CentralHighlandsofEthiopia

CIConfidenceInterval

CSA CentralStatisticalAgency

CSF ChaffaStateFarm

^{0}C DegreeCelsius

E.coliEscherichiaColi

EARNS EasternAmharaRegionalNationalState

ELISA EnzymeLinkedImmunoSorbentAssay

FAO FoodandAgriculturalOrganization

FPAFluorescencepolarizationassay

GBWOARDGudeyaBilaWoredaOfficeofAgricultureandRuralDevelopment

GSWOARDGobuSeyoWoredaOfficeofAgricultureandRuralDevelopment

IgA ImmunoglobulinA

IgG ImmunoglobulinG

IgM ImmunoglobulinM

µlMicroliter

MoAMinistryofAgriculture

NEE NorthEastEthiopia

NVINationalVeterinaryInstitute

ODOpticalDensity

OIE OfficeofInternationaldesEpizooties

ORODdRatio

PAHO PanAmericanHealthOrganization

PCR PolymeraseChainReaction

P-valueProbabilityvalue

RBPT RoseBengalPlateTest

RFM RetainedFetalMembrane

RLPSRoughLipopolysaccharide

S19Strain19

SEE SouthEastEthiopia

SLPSSmoothLipopolysaccharide

SPSSStatisticalPackageforSocialScience

SSWOARDSibuSireWoredaOfficeofAgricultureandRuralDevelopment

UKUnitedKingdom

USAUnitedStatesofAmerica

USDAUnitedStatesofDevelopmentAgency

WHO WorldHealthOrganization

ABSTRACT

Brucellosisisahighlycontagiousbacterialdiseaseofmajorsocio-economicandpublichealthimportancewhichcausedbygramnegativebacteriaofthegenus*Brucella*.Across-sectionalstudywasconductedoncattleinselecteddistrictsofEast-WollegazonebetweenNovember2018andSeptember2019toassessbovinebrucellosisseroprevalence,potentialriskfactors,knowledge-attitudeandpracticeoffarmownersaboutbrucellosis.Thestudyzoneanddistrictswereselectedpurposively, whilepeasantassociation,herdandindividualanimalswereselectedrandomly.Atotalof488bloodsampleswerecollectedfrom362localbreedand126crossbreedcattleofabovesixmonthsofage. TheRBPTscreened11*Brucella*seropositiveoutof488(2.25%)(95%CI:0.94-3.5).TheRBPTpositiveserawerefurtherretestedbyusingC-ELISAand6(1.23%)(95%CI:0.25-2.2)wereconfirmedtobeseropositive.Outof87herdsincludedinthestudy,6(6.9%)(95%CI:3.2-14.2)wereseropositiveusingC-ELISAwithatleastoneseropositiveanimalintheherd.Theoverallseroprevalenceofbrucellosiswas1.23%and6.9%atanimalandherdlevelrespectively.Moreover,informationwasgatheredonindividualanimalandfarmtoassessriskfactorsusingasemi-structuredquestionnairepreparedforthispurpose.Statisticalanalysiswasperformedusing SPSS version20softwareprogram.Theresultofmultivariablelogisticregressionanalysisshowedthatherdsize(OR:8.5,95%CI:1.217-19.872,P=0.031),age(OR:6.5:95%CI:1.459-28.967,P=0.014),pregnancystatus(OR:12.78,95%CI:2.35-45.725,P=0.009)andabortioncase(OR:8.3,95%CI:6.759-10.389,P=0.001)werethesignificantriskfactorsfor*Brucella*seropositivity.Theresultsofquestionnairesurveyrevealedthatthemajorityofthefarmownersorrespondentsdonothavesufficientknowledgeaboutbrucellosisandareatriskofacquiringtheinfection.Althoughtheoverallprevalenceofbovinebrucellosiswaslowinstudyarea,itcouldserveassourceofinfectiontodifferentherdsastherewerefociofinfectioninherdsandbrucellosisishighlycontagiousdisease.Hencebettercontrolandpreventionmeasuresshouldbeimplementedtoreduceriskofinfectionandtransmissionofthediseaseinlivestockandhumaninthestudyarea.

Key-words:*Bovine,Brucellosis,East-Wollega,Ethiopia,Oromia,Riskfactors,Seroprevalence.*

1.INTRODUCTION

1.1.Background

Sincetheearliestdaysofcivilization,maniscloselyassociatedwithanimalsandthusgaveanopportunityofintercommunicabilityofmicrobialinfectionsbetweenhumansandanimals(Radiostitis *etal.*,2007).Althoughmanyresearchesandinitiativeshavebeencarriedoutbyvariousnational,regionalandinternationalpublichealthagenciestoreducetheburdenofinfectiousdiseases,stillemergingandre-emerginginfectiousdiseasesposegreatthreatsandchallengestopublichealthworldwide(OlanoandWalker,2011;Birhan*etal.*,2015).Ofthesediseases,60%thataffecthumanhavezoonoticbackgroundsimplybecausehumanlifeisdependentoninteractionswithothercreatureslikelivestock(Megersa*etal.*,2011;Molyneux*etal.*,2011).

EthiopiahasoneofthelargestlivestockpopulationsinAfrica(CSA,2012/2013).Livestockcontributesmorethan30%oftheagriculturalgrossdomesticproductand19%inexportearnings(MoA,2012).Livestockalsoplaysacrucialroleintheeconomiesofmanydevelopingcountries.Theyprovidefood,ormorespecificallyanimalproteininhumandiets,income,employmentandpossiblyforeignexchange.Forlowincomeproducers,livestockalsoplaysasastoreofwealth;providedraughtpowerandorganicfertilizerforcropproduction.Thecomparativelyhugelivestockresourcesofthecountrytheeconomicreturngainedfromthissubsectordonotcoincide,becauseofprevalentinfectiousdiseases,amongotherfactors(Yifat*etal.*,2012).

Brucellosisisoneoftheseinfectious,contagious,andworldwidespreadformsofanimportantzoonoticdiseasecausedbybacteriaofthegenus*Brucella*.Brucellosisispublichealthproblemwithadversehealthimplicationsbothforanimalsandhumanbeingsaswellaseconomicimplicationsforindividualsandcommunities.Itisofmajorpublichealthimportanceinmostdevelopingcountries,whichhavenonationalbrucellosiscontrolanderadicationprogram(Radostits*etal.*,2007).Inadditionthepolicyofmanydevelopingcountries,importingexotic,highproductionanimals,withouthavingtherequiredveterinaryinfrastructureandappropriatelevelofdevelopmentofsocio-economicsituationsoftheanimalholdersaggravatesthesituation(Robinson,2003).

Millionsofindividualsareatriskworldwide,especiallyincountrieswhereinfectioninanimalshasnotbeenbroughtundercontrol,proceduresforheattreatmentofmilksuchaspasteurizationarenotroutinelyapplied,andstandardsofhygieneinanimalhusbandryarelow(Addis,2015).Consumptionofunpasteurizedrawmilkanddairyproductsisacommonmethodoftransmission(OIE,2009).

Theburdenofbrucellosisismainlyonthepoorindividualsastheyareoftenforcedtoliveinclosecontactwiththeiranimalsandsoaremorelikelytobecomeinfected(Raclozetal.,2013;Haghietal.,2015).Thediseaseresulttoprolonghealthproblemswhichmaycausepermanentdisabilitiesandisanimportantcauseoftravelassociatedmorbidity(Zinsstagetal.,2007).

Brucellosisincattleisprimarilycaused*byBrucellaabortus*,occasionallyby*Brucellamelitensis*andrarelyby*B.suis*whentheysharepastureorfacilitiescommonlywithinfectedpigs,goats,orsheep(Godfroidetal.,2013).Occasionallyotherspeciesofanimalssuchassheep,swine,dogsandhorsesmaybeinfectedwith*B.abortus*(Chauhanetal.,2017).

Bovinebrucellosisisaninfectiousdiseaseknownforitsimpactonreproductiveperformanceofcattleinafrica(McDermottandArimi,2002).Itisaneconomicallysignificantdiseaseoflivestockcausingreproductivewastagethroughinfertility,delayedheat,lossofcalves,reducedmeatandmilkproduction,cullingandeconomiclossesfrominternationaltradebans(OIE,2009).Themostcommonrouteoftransmissionincattleisthroughdirectcontactwithanabortingcowandtheabortedfetus.Ingestionofcontaminatedpastureandwatermayalsoplayasasecondaryrole(Robinson,2003).

Bovinebrucellosishasbeenreportedfromseveralpartsofthecountry(Asmareetal.,2010).Althoughthediseasehasbeeneradicatedfrommostofthedevelopedcountries,itisstillamajorpublicandanimalhealthprobleminmanydevelopingcountries,wherelivestockareamajorsourceoffoodandincome(Pappasetal.,2006).Thehighprevalenceisprobablyduetothefactthatmanycountrieshavenotyetstartedcontroloreradicationschemes(Alverazetal.,2011).BrucellosisisendemicinEthiopiasince1970(Yohannis,2017).Sincethen,studieshavedemonstratedthepresenceofantibodiesagainst*Brucella*inanimalsandhumansindifferentpartsofthecountry(Bekeleetal.,2000; Ibrahim *et al*., 2010;Degefaetal.,2011;Yohannesetal.,2013).

1.2.StatementoftheProblem

Brucellosishasconsiderableimpactonanimalandhumanhealth,aswellaswidesocio-economicimpacts,especiallyincountriesinwhichruralincomerelieslargelyonlivestockbreedinganddairyproducts(Maadietal.,2011).Livestockprovidesalifelineforalargeproportionof95%oftheworld'sruralpopulationthatliveinthedevelopingworld(Wadoodetal.,2009).Itcausesloss esduetoabortionorbreedingfailureintheaffectedanimalpopulation,diminishedmilkproduction andcausingreducedworkcapacitythroughsicknessoftheaffectedhuman(Bashituetal.,2015).

Ethiopiaisparticularlyvulnerabletotheeffectofzoonoticdiseasesbecausetheeconomyislargely dependentonagriculture(McDermott*etal.*,2013)andmajorityofhouseholdshavedirectcontact withdomesticanimals,creatinganopportunityforinfectionandspreadofdisease.InEthiopia,bru cellosisisfoundintopfivezoonoticdiseasesnexttorabiesandanthrax(Pieracci*etal.*,2016).

About85%oftheherdsinthestudyareasharedthecommunalgrazingsystem.Freegrazingallowsu nrestrictedcontactbetweenanimalsthatcontributesthespreadofbrucellosisinextensivemanage mentsystem.Theprevalenceislinkedtothepracticeofanimalmovementtocommunalwateringp ointsandotherareaswhensearchingforpastureandwater(Abubakar*etal*.,2012).

MostofthestudiesoncattlebrucellosishavebeencarriedoutincentralandnorthernEthiopiawhich focusedondairycattle'sofurbanandper-urbanareas((DinkaandChala,2009;Megersa*etal*.,2011).However,themajorityoflivestockwer efoundinruralareaswheremosthouseholdshavedirectcontactwithdomesticanimalsandthehabi tofconsumingrawmilk,raworundercookedmeatisstillacommonpractice,especiallyamongrura lcommunities(Kambarage*etal*.,2003;Shirima*etal*.,2003).Thiscouldmainlybeattributedtolack ofknowledgeofthezoonoticrisksassociatedwiththeconsumptionofunpasteurizedmilk.

ThereportsfromsomepartsofEthiopiaareindicatingthattheoccurrenceoflivestockandhumanbr ucellosisisincreasing(DinkaandChala,2009).However,itisdifficulttonotethegeneralprevalen ceofanimalandhumanbrucellosisinthewholecountryduetolackofuniformstudiesindifferentpa rtsofthecountry.Thelimitedstudies(surveys)sofarconductedonbrucellosisarenotsufficienttos howtheexactnationalpictureandsignificanceexcepthighlightingtheexistenceofthediseaseinv erylimitedareasofthecountry.

ThereisinadequateinformationonthestatusofbovinebrucellosisinEastWollegazoneofOromia region,westernEthiopia((DinkaandChala,2009;Megersa*etal*.,2011).Theprevalenceofbrucell osisincattleinselecteddistrictsofEastWollegazonealsohasnotbeenstudiedyet.Thereforethisst udyhasbeenconductedwiththefollowingobjectives**.**

- Toassesstheoverallsero-seroprevalenceofbovinebrucellosisinstudyarea.
- Toassesspotentialriskfactorsof bovinebrucellosisinthestudyareas.
- Toassessknowledge-attitudesandpracticesoffarmownersaboutbrucellosis.

2.LETIRATUREREVIEW

2.1.TheCausativeAgent

Thegenus*Brucella*resideswithinthefamily*Brucellaceae*,orderRhizobiales,classAlphaproteobacteriaandphylumProteobacteria.Allproteobacteriaaregram-negative,withanoutermembranemainlycomposedoflipopolysaccharides(MurrayandHolt,2005).*Brucella*issmallgram-negativebacteria,coccobacilli,non-motile,non-sporulating,non-toxigenic,non-fermenting,facultativeintracellularorganism,thatcaninfectmanyspeciesofanimals,includinghumans(Manture*tal*.,2007).Thecellularandcolonialmorphologyofthe*Brucella*speciesareuniformorsimilarinmostrespect.All*Brucella*speciespossesssmoothlipopolysaccharide(SLPS)intheiroutercellwallexcept*B.ovisandB.canis*,whichhaveroughlipopolysaccharide(RLPS)andprot

einantigens(Lapaque*etal*.,2005).

Todate,tenspeciesarerecognizedwithinthegenus*Brucella*(Godfroid*etal*.,2011).Thegenus*Bru cella*consistsofsixclassicspeciesthatinfectlandanimalsnamely;*B.melitensis,B.abortus,B.suis ,B.ovis,B.neotomae*and*B.canis*.The*B.melitensis*biovars(bvs)1-3(mainlyisolatedfromsheepandgoats),*B.abortus*bvs1-6and9(fromcattleandotherbovidae),*B.suis*bvs1-3(frompigs),bvs4(fromreindeer)andbvs5(fromsmallrodents),*B.canis*(fromdogs),*B.ovis*(fro msheep)and*B.neotomae*(fromdesertwoodrats).Thisclassificationisbasedmainlyondifference sinpathogenicityandhostpreference(Moreno*etal*.,2002).

*Brucellaabortus*isthecausativeorganismforbovinebrucellosis.*Brucellaabortus*ismainlyinfec tiveforcattle,butoccasionallyotherspeciesofanimalssuchassheep,swine,dogsandhorsesmayb einfected.Although*Brucellaabortus*infectingcattlehassevenrecognizedbiovars,themostrepor tedofwhicharebiovars1,2,3,4,and9,withbiovar1beingthemostprevalent.Thedistributionofbio varscouldbeimportantinascertainingthesourceofsomeinfections(Neta*etal*.,2010).Cattlealsob ecomeinfectedby*B.suis*and*B.melitensis*whentheysharepastureorfacilitieswithinfectedpigs,g oats,orsheep.Theinfectionsincattlecausedbyheterologousspeciesof*Brucella*areusuallymoret ransientthanthatcausedby*B.abortus*(Bashitu*etal*.,2015).

Recently,severalnewmarinespecieshavebeendescribedincluding*B.innipedialis*(isolatedfrom seals)and*B.ceti*(isolatedfromwhalesandDolphins)(Foster*etal*.,2007),*B.microti*(isolatedfrom thecommonvoles(Microtusarvalis)andredfoxes(Vulpesvulpes)(Scholz*etal*.,2008;Scholz*etal* .,2009)andlastly*B.inopinata*(isolatedfromahumanbreastimplantwound)istheonlyspeciesthat hasnotbeenisolatedfromanyanimalreservoir(Scholz*etal*.,2010).

2.1.1.Resistanceandsurvivalproperties

Underfavorableconditions,*Brucella*organismscansurviveintheenvironmentforaverylongperi od.Theirabilitytowithstandinactivationundernaturalconditionsisrelativelyhighcomparedwit hmostothergroupsofnon-sporeformingpathogenicbacteria(Jegerfa*etal*.,2009).*B.abortus*issensitivetopasteurizationte mperaturesanditssurvivaloutsidethehostislargelydependentonenvironmentalconditionssuch asmoisturecontent,temperature,changesinpH,humiditylevelandconditionsofstorage.Inrawm ilk*Brucellae*cansurvivefor24hoursat25-37^{0}C,at8^{0}Ccansurvivefor48hourswhileat-40^{0}Ccansurvivefor2.5years.Thepathogenmaysurviveinabortedfetusintheshadeforuptoeight

months,fortwotothreemonthsinwetsoil,onetotwomonthsindrysoil,threetofourmonthsinfaeces,andeightmonthsinliquidmanuretanks(Yohannes,2017).

Survivalisprolongedwhenthetemperatureislow,particularlywhenitisbelowfreezing.Itshouldbenotedthatthebacteriaareparticularlysusceptibletoheatanddesiccationanddirectsunlightwillrapidlydestroyexposedorganisms.Carbondioxideisimportantelementsforgrowthof*Brucella*organism,especially*B.abortus*;suchorganisms,whichrequirecarbondioxidefortheirgrowth,arecalledcapnophilicorganisms.AtPH<4,*Brucella*agentsdonothavepotentialtosurvive(PadillaPoster*etal*.,2010).Allstandarddisinfectantsdestroy*Brucella*species.A10g/lsolutionofphenolwillkill*Brucella*afterlessthan15minutesexposureat37^{0}C.Formaldehydesolutionisthemosteffectiveofthecommonlyavailabledisinfectants(Yohannes,2017)

2.2GeographicalDistribution

2.2.1Globaldistributionofanimalbrucellosis

Brucellosishasworldwidedistribution,butnowadaysthediseaseisrareinmanyindustrializedordevelopednationsbecauseofroutinescreeningofdomesticlivestockandanimalvaccinationprograms.Althoughthedistributionofbrucellosisisworldwide,thediseaseismorecommonincountrieswithpoorlystandardizedanimalandpublichealthprograms(Roba,2017).Thisdisease,however,isaleadingcauseofzoonoticinfectionsandofeconomicimportanceinthecountriesoftheEasternMediterraneanRegion(Cadmus*etal.*,2013).

*Brucellaabortus*isfoundworldwideincattle-raisingregions,exceptinJapan,Canada,andsomeEuropeancountries.Australia,NewZealand,andIsraelareamongfewcountrieswhereithasbeeneradicated.Eradicationofdiseasefromdomesticatedherdsisalmostcompleteinthe USA.*B.abortus*canbefoundinwildlifeanimalsinsomeregions,includingtheGreaterYellowstoneAreaofNorthAmerica(Asmare,*etal*.,2010).

2.2.2Globaldistributionofhumanbrucellosis

BrucellosisisnamedafterSirDavidBruce,whoin1886isolatedthecausativeagentfromasoldierinMaltawherethediseasecausedconsiderablemorbidityandmortalityamongBritishmilitarypersonnel.Duringthe19thcentury,brucellosiswasthusknownasMaltaorMediterraneanfever(Buzgan*etal.*,2010).Humanbrucellosisisalsoknownbymanydifferentnamessuchasintermittenttyphoid,RockfeverofGibraltar,andmorecommonly,undulantfever(Buzgan*etal*.,2010).Humanbr

ucellosistendstooccurmorecommonlyinregionswithlessestablishedanimaldiseasecontrolprogramsandinareaswherepublic-healthinitiativesmaybelesseffective.Anestimated500,000newhuman*Brucella*caseswererepo rtedannuallyworldwide(Pappas*etal.*,2006).Fourspeciesof*Brucella*haveknownpathogenicity forhumansworldwide,theseinclude;*B.melitensis*,*B.abortus*,*B.suis*and*B.canis*(Godfroid*etal.*, 2011).However,*B.melitensis*,*B.arbotus*,and*B.suis*arehighlypathogenicforhumanswith*B.mel itensis*beingthemostpathogenicforhumans(OIE,2011).

HumanbrucellosisisknowntobehighlyendemicintheMediterraneanbasin,MiddleEast,Wester nAsia,AfricaandSouthAmerica(Pappas*etal.*,2006).Countrieswiththehighestincidenceofhum anbrucellosisincludeSaudiArabia,Iran,PalestinianAuthority,Syria,JordanandOman(Pappas*e tal.*,2005).Syriahadthehighestannualbrucellosisincidenceworldwide,reachinganalarming16 03casespermillionperyearaccordingtodatafromOIE(2004).IntheUnitedArabEmirates,mostc asesarereportedfromDubai,apopularinternationaltraveldestination,underliningtheimportanc eofthediseaseinthefieldoftravelmedicine(Refai,2002).IntheUnitedStates,brucellosisismuchl esscommon,withonly100-200humancasesreportedeachyear.ThisdecreaseincasesintheUnitedStatesisfelttobeduetoeffe ctiveanimalvaccinationprogramsandmilkpasteurization.InEurope,humanbrucellosisisthoug httobeassociatedwithtravellersandimmigrantsfromtheMiddleEastortheprivateimportofdairy productsfromendemicareas(Georgi*etal.*,2017).TheWorldBank(2011)rankedDubaiandAbu Dhabiasbeingthesecondandthird,mostpopularmedicaltourismdestinationintheregionbehindJ ordan(Refai,2002).

2.2.3Africaandthesub-regiondistributionofbovinebrucellosis

AccordingtotheOIE(2009)bovinebrucellosisisareportablezoonosisandisofconsiderablesocio economicconcern.MostAfricancountriesareofpoorsocioeconomicstatus,withpeoplelivingwi thandbytheirlivestock,whilehealthnetworksandsurveillanceandvaccinationprogramsarevirt uallynon-existentinmostAfrica(McDermottandArimi,2002).Inmostlow-incomecountries,thereismuchlesspublicinvestmentinveterinaryandhealthservices,withweak ersurveillanceandoperationalcapacity.Suchinterventionsarenotfeasibleinmanydevelopingco untriesbecauseofpoorsurveillanceprograms,limitedinstitutionalcapacityandlackoffundsforli vestockholdercompensation(Zinsstag*etal.*,2007).

Inmostsub-Saharancountries,cattleseroprevalenceestimateshavebeenobservedtorangebetween3and15%(Ghanem*etal*.,2009;Jergefa*etal*.,2009;Haileselassie*etal*.,2010).InAfrica,bovinebrucellosiswasfirstrecordedinZimbabwe(1906),Kenya(1914)andinSouthAfricaintheyear1915(Chukwu,1985).However,stilltheepidemiologyofthediseaseinlivestockandhumansaswellasappropriatepreventivemeasuresarenotwellunderstoodandsuchinformationisinadequateparticularlyinsubSaharanAfrica.

Table1.DistributionofbovinebrucellosisinsomeAfricancountries

Country	Host	Notested	Prevalence (%)	Tests used	Reference
Eretria	Cattle	15049	2.77	CFT	Scacchia*etal*.,2013
Zambia	Cattle	395	20.7	c-ELISA	Muma*etal*.,2013
Sudan	Cattle	250	2	ELISA	SeneinandAbdelkadir,2012
Kenya	Cattle	393	1	c-ELISA	Kang‟ethe*etal*.,2007
Zimbabwe	Cattle	1291	5.5	c-ELISA	Matope*etal*.,2010
Somaliland	Cattle	153	1.96	RBPT	Ahmed,2009
Nigeria	Cattle	220	5.45	RBPT	Bwala*etal*.,2015
Tanzania	Cattle	655	5.3	RBPT	SwaiandSchoonman,2010
Uganda	Cattle	423	5	c-ELISA	Makita*etal*.,2011
Gambia	Cattle	465	1.1	CFT	Unger*etal*.,2003
Senegal	Cattle	479	0.63	CFT	Unger*etal*.,2003
Ghana	Cattle	444	2.93	RBPT	Folitse,2014

Cameroon	Cattle	940	9.64	i-ELISA	Shey-Njila,2005
Djibouti	Cattle	428	4	RBPT	Chantal*etal.*,1994

2.2.4.TheStatusofbovinebrucellosisinEthiopia

Eventhough,severalserologicalsurveyshaveshowedbovinebrucellosisisanendemicandwidespreaddiseaseinEthiopia,mostofthestudiesoncattlebrucellosishavebeencarriedoutincentralandnorthernEthiopiaanddonotprovideanadequateepidemiologicalpictureofthediseaseindifferentagro-ecologicalzonesandlivestockproductionsystemsofthecountry((DinkaandChala,2009;Megersa*etal*.,2011).Theproblemiscompoundedbyanabsenceofofficiallycoordinatedprogramforcontrolofdisease,surveillanceprograms,diagnosticfacilitiesorreliabledata.

TheevidencesofbrucellosisinEthiopiancattlehavebeenserologicallydemonstratedbydifferent authors.Mostofthestudiessuggestedalowseroprevalence(below5%)incattleundercrop-livestockmixedfarming(Berhe*etal*.,2007;Ibrahim*etal*.,2010;Adugna*etal*.,2013).Theevidencesof*Brucella*infectionsinEthiopiancattlehavebeenserologicallyevaluatedindifferentpartsofthecountrybydifferentauthorsindifferentproductionsystem.

Sincethefirstreportofbrucellosisinthe1970sinEthiopia,thediseasehasbeennotedasoneoftheimportantlivestockandhumandiseasesinthecountry(Ibrahim*etal*.,2010;Tesfaye*etal*.,2011;Geresu*etal*.,2016).InEthiopia,informationonlossesspecificallythroughbrucellosisinthedifferenttypesofproductionsystemsissparse,withtheexceptionofSintaro(1994)whoreportedanannuallossfrombrucellosisestimatedtobe88,941.96EthiopianBirr($5231equivalent)among193cattle,largelyduetoreducedmilkproductionandabortions(ChaffaStateFarm,Wollo,from1987to1993).

Prevalenceinintensivemanagementsystem

Higherindividualbovinebrucellosisseroprevalencehasbeenrecordedinintensivelymanagedcattleherdsascomparedtothoseintheextensivemanagementsystem.InBorenazoneofOromiaregion,thehighestseroprevalence(50%)wasdocumentedusingELISAinDidituyuraranch(AlemandSolomon,2002).Aseroprevalenceof39%wasalsorecordedattheInstituteofAgriculturalResearchinWesternEthiopia(Meyer,1980),22%indairyfarminNortheasternEthiopia(Sintaro,1994),11to15%indairyfarmsandranchesinSoutheasternEthiopia(Bekele*etal*.,2000),and7.7%inTigrayregion(Haileselassie*etal*.,2010).

Relativelylowindividualanimalseroprevalencewererecordedinsomeintensivefarmsindifferentpartsofthecountry.Tolosa(2004)documented1.7%inJimmaZoneofSouthernEthiopia,Kassahun*etal*.(2007)documented2.46%inSidamaZoneofSouthernEthiopia;Mussie(2007)reportedaprevalenceof0.26%inWesternpartofAmharaRegionalState;Bashitu*etal*.(2015)reportedaprevalenceof0.2%indairycattleofDebrebirhanandAmboTowns.Accordingtotheseauthors,thereasonsforthelowprevalenceofbovinebrucellosisinthesestudyareaswereexplainedbybetterhygienicpractices,useofmaternitypenand/orseparationofcowsduringparturition,cleaninganddisinfectionactivities,cullingofinfectedanimalsdependingonownherdsforreplacingstockandfarmownersknowledgeofbrucellosisintheseintensivefarms.

Prevalenceinextensivemanagementsystem

InEthiopia,95%ofcattlearefarmedunderextensivesystems.Accordingtotheavailabledata,*Brucella*seroprevalencewithinextensivecattlerearingsystemsislowerthanthatofintensivesystems.ReportsfromNorthTigrayregion(Haileselassie*etal*.,2010)andSouthernSidamazone(Asmare*etal*.,2010),anoverallprevalenceof1.2and1.66%wererecordedfollowingscreening848and1627cattlefromextensivesystem,respectively.Across-sectionalepidemiologicalstudycarriedoutinTigrayRegionofEthiopiarevealedthatof816indigenouscattleseraexamined,only27(3.3%)wereseropositiveusingRBPT,ofwhich26(3.19%)werealsopositivebyCFT.Overallherd-levelprevalencewasreportedtobe42.31%andthewithin-herdprevalencevariedfrom0to15.15%basedonCFT(Berhe*etal*.,2007).Inanotherstudy,Ibrahim*etal*.(2010)reportedoverallindividualandherdlevelseroprevalencesof3.1and15.0%,respectively.UsingCFT,Kebede*etal*.(2008)reportedindividualandherdanimalprevalenceof11%and45.9%,respectively.DinkaandChala(2009)investigatedbovinebrucellosisusingRBPTinfourdistrictsofEastShowaZone.Intheirstudy,*Brucella*antibodywasdetectedin8.7,18.6,5.1and10%ofthesamplesinFentale,ArsiNegele,LumeandAdamiTulustudydistrictsrespectively.Theoverallherdprevalencewasreportedtobe11.2%.Jergefa*etal*.(2009)alsoconductedseroprevalencestudyusingRBPTandCFTinthreeagroecologicalareasofcentralOromianamely:Walmara,Adami Tulu-JidoKombolchaandLumeDistricts.Theirresultrevealedoverallprevalenceof2.9and13.6%inindividualanimalandherdlevel,respectively.Adugna*etal*.(2013)reportedoverallanimallevelseroprevalenceof1%incattleunderatraditionalproductionsysteminWesternEthiopia.RecentlyYoh

annes,(2017)reportedoverallindividualanimalprevalenceandherdprevalenceof1.3%and5.8%,respectivelyincattlefarmedunderextensivesystemsinWolaitaZone.

2.3.PossibleRiskFactorsforInfection

2.3.1.Animalriskfactors

Susceptibilityofcattleto*B.abortus*infectionisinfluencedbytheage,sexandreproductivestatusoftheindividualanimal.Sexuallymaturepregnantcattlearemoresusceptibletoinfectionwiththeorganismthansexuallyimmaturecattleofeithersex.Susceptibilityincreasesasstageofgestationincreases(Tsegaye*etal.*,2016).Mostanimalsinfectedasadultsremaininfectedforlife.Herdsizeandanimaldensityaredirectlyrelatedtoprevalenceofdiseaseanddifficultyincontrollinginfectioninapopulation(Radostits*etal*.,2006).

2.3.2.Pathogenriskfactors

*Brucellaabortus*isafacultativeintracellularorganismcapableofmultiplicationandsurvivalwithinthehostphagocyticcells.Theorganismsarephagocytizedbypolymorphonuclearleucocytesinwhichsomesurviveandmultiply.Theorganismisabletosurvivewithinmacrophagesbecause;ithastheabilitytosurvivephagolysosome.Thebacteriumpossessesanunconventionalnonendotoxinlipopolysaccharidewhichconfersresistancetoantimicrobialattacksandmodulatesthehostimmuneresponse.Thesepropertiesmakelipopolysaccharideanimportantvirulencefactorforsurvivalandreplicationof*Brucella*(Ramirez*etal*.,2006).

2.3.3.Occupationalriskfactor

Laboratoryworkershandling*Brucella*culturesareathighriskofacquiringbrucellosisthroughaccidents,aerosolizingand/orinadequatelaboratoryprocedures.Inadditiontothis,abattoirworkers,farmersandveterinariansareathighriskofacquiringtheinfection(Chain*etal*.,2005).

2.3.4.Managementriskfactors

Thespreadofthediseasefromoneherdtotheotherandfromoneareatoanotherisalmostalwaysduetothemovementofaninfectedanimalfrominfectedherdintoanon-infectedsusceptibleherd(Addis,2015;Tsegaye*etal.*,2016).Largenumbersoforganismsareshedfromthereproductivetractwheninfectedcowsabort.Incowswhichlactatefollowingabortion,milk,includingcolostrum,isanimportantsourceofinfection,andbacteriaareexcretedintermittentl

yinmilkthroughoutthelactationperiod.Thefluidinhygromascausedby*Br.abortus*infectionma ycontainlargenumbersoforganisms,butbecauseofbeingrestrictedtothelesiontheydonotseemt obeimportantinthespreadofthedisease(Tolosa,2004).

2.4.TransmissionofBrucellosis

2.4.1Transmissionofbrucellosisinanimals

Incattle,transmissionof*B.abortus*typicallyoccursthroughingestionoflivebacteria.Itistransmit tedamonganimalsmainlythroughingestionofcontaminatedfeedandwaterandoccasionallybyi nhalationofaerosolsorbydirectcontactwithinfectedmaterials(McDermottandArimi,2002;Ma urin,2005).

Themostsignificantfeatureofbovinebrucellosisepidemiologyisthesheddingoflargenumberso forganismsduringthetendaysafterabortionorcalvingofinfectedcowsandtheconsequentconta minationoftheenvironment.Thediseasespreadthroughcontaminationofplacentalmaterialand vaginaldischargesofabortinganimal(Abubakar*etal*.,2012).

Movementofinfectedcattleintoaherdcanresultintransferofthediseasewhencattleingestthebact eriafromabortedfetuses,placentaanddischargesfromcowsthathaveabortedorcontaminatedpas tureorwater(Park*etal*.,2005).Venerealtransmissionsbyinfectedbreedingbullstosusceptibleco wsappeartoberare.Transmissionmayoccurbyartificialinseminationwhen*Brucella*contaminat edsemenisdepositedintheuterusbutreportedlynotinmidcervix(Cheville*etal.*,1998).Venereal transmissionisanimportantrouteofspreadinpigs(Poester*etal.*,2013).Theincubationperiodvarie swidelydependingonexposuredose,previousvaccination,species,age,sexandgestationofpreg nancy(NicolettiandGilsdorf,1997).Thetransmissionofbrucellosisbyticks,fleasormosquitoesf romaninfectedherdtonon-infectedherdhasneverbeenproved(OIE,2009).

2.4.2.Transmissionofbrucellosisinhumans

Thediseaseismainlytransmittedtohumansthroughingestionofcontaminatedanimalproductssu chascheeseandunpasteurizedmilkandbydirectcontactwithinfectedanimalsthroughhandlinga bortions,dystociaandparturitions(Shirima*etal.*,2010).Thesourceofnaturallyacquiredbrucello sisinhumansisalmostalwaysfromanimalreservoirs,butveryfewcasesofhumantohumantransm issionviabloodtransfusion,intrauterineinfection,organandtissuetransplantation,sexualcontac t,andbreastfeedinghavebeenreported(Godfroid*etal.*,2011).Thesourceofhumaninfectionresid

esalwaysindomesticorwildanimalreservoirs.Theriskofcontractingzoonosisfromwildlifeishigherinpoorcommunitieswhosepeopleandlivestockinteractwithwildlife,commonlyreferredtoaswildlife-livestockinterfaceareas(Mumaetal.,2014).Wildlife-livestockinterfacesposeachallengetohuman,animalandenvironmentalhealthpractitionersduetothecomplexandcontinuouscycleofdiseasetransmission(Pandeyetal.,2013).

Fromthepublichealthviewpoint,brucellosisisconsideredtobeanoccupationaldiseaseforpeoplewhoworkwithinfectedanimals,particularlyfarmworkers,veterinarians,ranchers,gamehuntersandmeatpackagingfactoryemployees(OIE,2011).Humaninfectiontransmissiontypicallyoccursthroughthreeprimarysourceswhichinclude;consumptionofunpasteurizeddairyproductswherebrucellosisisendemic,contactwithinfectedlivestockorwildanimals,meatortissuesofanimalsandlaboratoryexposures.Infectionmayalsooccurbyinhalation,conjunctivalcontamination,accidentalingestion,skincontaminationespeciallyviacutsandabrasionandaccidentalself-inoculationwith*Brucella*S19vaccineduringfieldvaccinationcanleadtobrucellosistransmissiontohandlers(WHO,2006).

*Brucella*ishighlyinfectiousinlaboratorysettingsandnumerouslaboratoryworkerswhoculturetheorganismhavebecomeinfected.Itisafrequentlyreportedlaboratoryacquiredinfection(Singh*etal.*,2015).*Brucella*organismscanbeshedinthemilkofinfectedanimalsforvariablelengthoftime,butformany,itcanbeshedforthelifeoftheinfectedanimal(MerckVeterinaryManual,2012).Although*Brucella*agentscanbetransmitteddirectlyandindirectlyfromitsanimalreservoirtohumans,indirecttransmissionremainsthehighestoverallriskandmainlyoccursthroughtheconsumptionofunpasteurizedmilkordairyproducts(Godfroid*etal.*,2005).Freshmilkanddairyproductspreparedfromunpasteurizedmilksuchassoftcheeses,yoghurtsandicecreamsmaycontainhighamountsofthebacteriaandconsumptionoftheseisanimportantcauseofhumanbrucellosis(Makita*etal.*,2008).

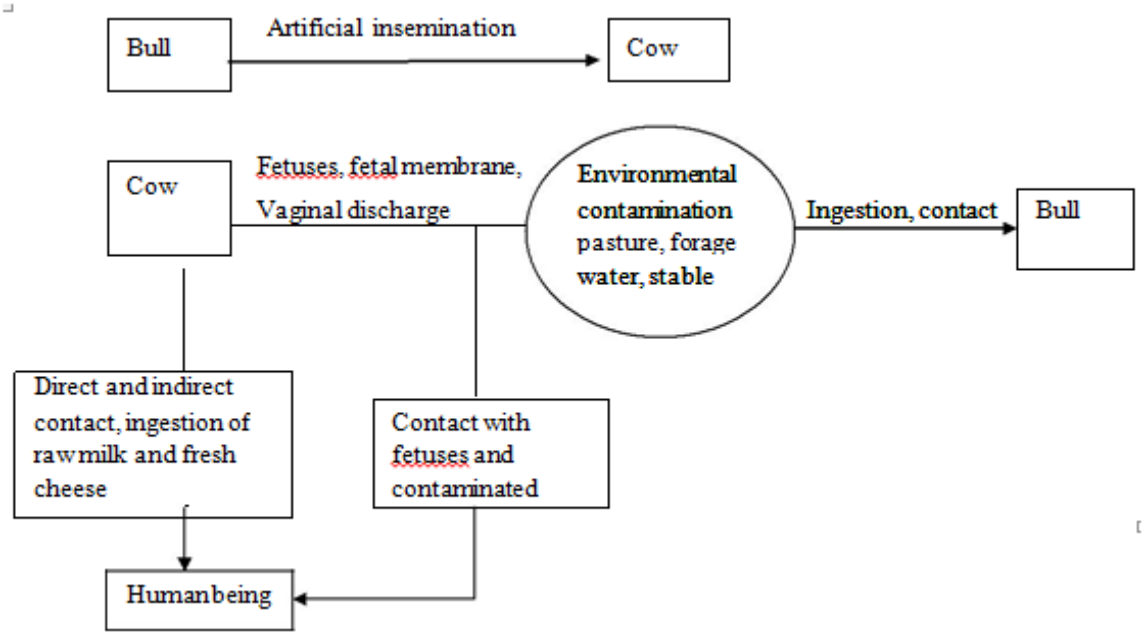


Figure1:Modeoftransmissionofbovinebrucellosis(*B.abortus*)

Source:AchaandSzyfres,2001

2.5.Pathogenesis

Theabilityofthepathogentosurviveandreplicatewithindifferenthostcellsexplainsitspathogenicity.Pathogenesisdependsuponvariousfactorssuchasthe*Brucella*species,sizeordoseoftheinoculum,modesoftransmissionandtheimmunestatusofthehost(Muflihanah*etal*.,2013).*Brucella*entersthebodyviatheingestion,conjunctivalmucosa,respiratorytract,orskinandafterinitialinvasionofthebody,localizationoccursinitiallyinthelymphnodes.Virulent*Brucellae*havetheabilitytosurviveinbothpolymorphonuclearandmononuclearphagocytesandalsocandepresschemotaxisandphagocytosisbypolymorphonuclearleucocytes(James,2013).

*Brucella*multipliesinthelymphnodesasparasitesandthenenterstheblodandproducesthebacteraemiafollowedbytheacutefebrilephaseofthediseaseafterphagocytosis.Fromtheblood,theorganismsaredistributedthroughoutthereticuloendothelialsystemandbecomepresentinmanyothersites(James,2013).

*Brucellaabortus*haspredilectioninthepregnantuterus,udder,testicleandaccessorymalesexglands,lymphnodes,jointcapsuleandbursa.Iftheinfectedanimalsarepregnant,*B.abortus*willcolonizeandreplicateinhighnumberinthechorionictrophoblastofthedevelopingfetus.Thepreferentiallocalizationtothereproductivetractofthepregnantanimalsisduetothepresenceofunknownfac

torsinthegraviduterus.Thesearecollectivelyreferredtoasallantoicfluidfactorsthatwouldstimulatethegrowthof*Brucella*.Erythritol,afour-carbonalcohol,isconsideredtobeoneofthesefactors(Pandey*etal.*,2013)whichareelevatedintheplacentaandfetalfluidfromaboutthefifthmonthofgestation(Yohannes,2017).Thepreferentialreplicationof*Br.abortus*intheextraplacentomalsitewithintrophoblastsofthechorioallantoicmembraneresultsinruptureofthecellsandulcerationofthefetalmembrane.Thedamagetoplacentaltissuetogetherwithfetalinfectionandfetalstresswillinducematernalhormonalchanges.Asaresult,abortionoccursprincipallyinthelastthreemonthsofpregnancy.Theincubationperiodisinverselyproportionaltothestageofdevelopmentofthefetusatthetimeofinfection(Megid*etal.*,2010).

2.6.ClinicalSigns

2.6.1.Clinicalsignsinanimals

Theincubationperiodvariesbetween14and120days(Radiostitis*etal.*,2000).Primaryclinicalmanifestationsofbrucellosisamonglivestockarerelatedtothereproductivetract.Incattle,*Babortus* causesabortions,stillbirthsandweakcalves.Infectionsinnon-pregnantfemalesareusuallyasymptomatic,butpregnantadultfemalesinfectedwith*Babortus*developplacentitis,whichnormallycausesabortionbetweenthefifthandninthmonthofpregnancy.Theplacentamayberetainedandlactationmaybedecreased.Epididymitis,orchitisandtesticularabscessesaresometimesseeninbulls(Cadamus*etal*.,2006).Infertilityoccursoccasionallyinbothsexes,duetometritisororchitis/epididymitis.Hygromas,particularlyonthelegjoints,areacommonsymptominsometropicalcountries.Arthritiscandevelopafterlong-terminfections.Systemicsignsdonotusuallyoccurinuncomplicatedinfections,anddeathsarerareexceptinthefetusornewborn.Femalesusuallyabortonlyonce,presumablyduetoacquiredimmunity(Yohhannes,2017).Evenintheabsenceofabortion,thereisheavyshеddingofbacteriathroughtheplacenta,fetalfluidsandvaginalexudates(OIE,2010).

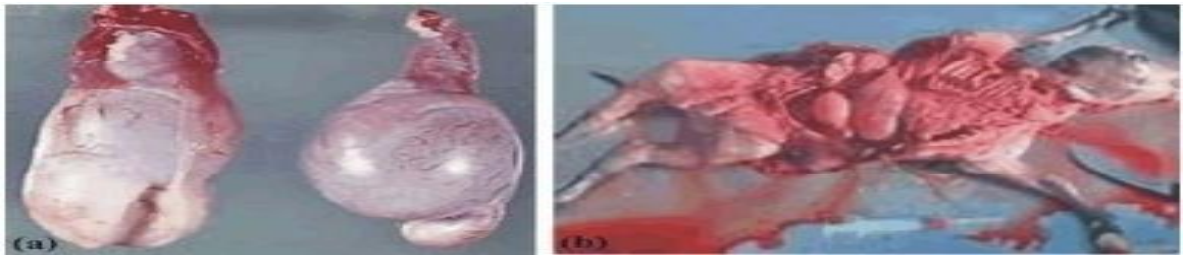


Figure2:Epididymitisinbulls(a)andabortionincow(b)

Source:AchaandSzyfres,2001

Figure3:Hygromasonlegjoints

Source:Godfroid*etal*.,2004

2.6.2.Symptomsofhumanbrucellosis

Themostcommonsymptomsofhumanbrucellosisincludeundulantfeverinwhichthetemperaturecanvaryfrom37.8^{0}Cinthemorningto40^{0}Cintheafternoon;nightsweetsandweakness.Commonsymptomsalsoincludeinsomnia,anorexia,headache,constipation,sexualimpotence,nervousness,encephalitis,arthritis,endocarditis,orchitisanddepression(Quinn*etal*.,2002).Spontaneousabortionseenmostlyinthesecondandthirdtrimestersofpregnancyinpregnantwomeninfectedwith*Brucella*.Lackofappropriatetherapyduringtheacutephasesmayresultinlocalizationof*Brucella*invarioustissuesandorgansandleadtosub-acuteorchronicdiseasewhichisveryhardtotreat(Bosilkovski*etal*.,2007).

2.7.Diagnosis

Clinicianmustdevelopahighdegreeofclinicalsuspicionbasedonepidemiologicalinformationandhistorywhicharecriticaltomakingtheclinicaldiagnosis.Inallcasesasampleshouldbecollectedfromthepatientandlaboratorytestingshouldberequestedasthedefinitediagnosisofbrucellosisisimpossiblewithoutlaboratoryconfirmation(Bricker,2002).Inmostdevelopingcountries,surveillanceofzoonoticdiseasesisnotrecognizedasa“onehealth”collaborationundertakingbetwee

nveterinarymedicineandhumanmedicine.Inaddition,manycountrieslackdiagnosticcapacityandhealthinfrastructuretodiagnosethedisease(Muma*etal.*,2014).Despitethevigorousattemptsformorethanonecenturytocomeupwithadefinitivediagnostictechniqueforbrucellosis,diagnosisstillreliesonthecombinationofseveralteststoavoidfalsenegativeandpositiveresults(Poester*et al.*,2010).Severaldiagnosticmethodshavebeenusedinthediagnosisofbrucellosis,theseincludes;bacteriologicaldetectionmethods,directlydemonstrationofantibodiesusingserologicaltechniquesandmolecularmethods(James,2013).

2.7.1.Bacteriologicaldetectionmethods

Theisolationandidentificationof*Brucella*offersadefinitivediagnosisofbrucellosisandusefulforepidemiologicalpurposes.Itshouldbenotedthatallinfectedmaterialspresentaserioushazard,andtheymustbehandledwithadequateprecautionsduringcollection,transportandprocessing.Apresumptivebacteriologicaldiagnosisof*Brucella*canbemadebymeansofthemicroscopicexaminationofsmearsfromvaginalswabs,placentasorabortedfoetuseswiththeStampmodificationoftheZiehl-Neelsenstainingmethod.However,morphologicallyrelatedmicro-organisms,suchas*Chlamydophilaabortus,Chlamydiapsittaci*and*Coxiellaburnetti*canmisleadthediagnosisbecauseoftheirsuperficialsimilarity(Poester*etal.*,2010).Accordingly,theisolationof*Brucella*speciesonappropriateculturemediasuchasFarrell'sselectivemediaisrecommendedforanaccuratediagnosis(Marín*etal.*,1996).

Isolationmaybeperformedbyculturingbodytissuesorsecretionslikeblood,milkandvirginaldischarge(Poester*etal.*,2010).*Brucella*speciescanalsobeculturedfrompus,jointandasciticfluids.Vaginalswabsandmilksamplesarethebestsamplestouseinisolating*Brucella*fromanimals(Roba,2017).Theidentificationof*Brucella*speciesinculturedependsonagreatdealofphenotypictraitssuchas:CO2requirementandbiochemicaltests(Bricker,2002).Brothoragarcanbepreparedfrompowdermediaforcultureof*Brucella*organisms.Duetothelow*Brucella*loadinthebloodandmilk,brothorabiphasicmediumisrecommendedforimprovingsensitivity(Poester*etal.*,2010).However,forotherspecimens,solidmediasuchasdextroseagar,tryptoseagar,andtrypticasesoyagar,arerecommendedforprimaryisolationof*Brucella,*butsomespecies,i.e.,*B.ovis*and*B.canis*requireadditionof5-10%ofsterilebovineorequineserumtotheculturemedia.OptimumpHforgrowthof*Brucella*variesfrom6.6to7.4,andculturemediashouldbeadequatelybufferednearpH6.8foroptimumgrowth.Theoptimumgrowthtemperatureis36-38°C.However,moststrainsgrowbetween20and40^{0}C(Poester*etal.*,2010).

ThemostwidelyusedselectivemediumistheFarrell'smedium(Marin*etal.*,1996),whichispreparedbytheadditionofsixantibioticstoabasalmediumtoinhibitgrowthofcontaminantsthatmaypreventisolationof*Brucella*species.Onsuitablesolidmedia,*Brucella*coloniescanbevisibleafter2–3-daysofincubation.After4daysofincubation,*Brucella*coloniesareround,1–2mmindiameter,withsmoothmargins.Theyaretranslucentandapalehoneycolourwhenplatesareviewedinthedaylightthroughatransparentmedium(OIE,2012).

InoculationintoGuineapigandmouseisanothertechniquethathasvaluefortheisolationof*Brucella*whenspecimensarederivedfrompotentiallycontaminatedsourcessuchasmilk,cheese,semen,orgenitaldischarges.Inoculationshouldbemadesubcutaneouslyintoguineapigorintravenously(0.1ml),orsubcutaneouslyifthematerialisheavilycontaminated,intomice.Aguineapigiskilled3weekspostinfectionand6weeksafterinoculation(Poester*etal*.,2010).

2.7.2.Serologicaldiagnosis

Serologicaltestsarerelativelyeasytoperformandprovideapracticaladvantageindetectingtheprevalenceof*Brucella*infection.Thetestsarecrucialforlaboratorydiagnosisofbrucellosissincemostofcontrolanderadicationprogramsrelyonthesemethods.Despitethedevelopmentofnumerousserologicaltests,nosingletestidentifiesallinfectedanimalsandawidevariationexistsinestimatesoftheirdiagnosticaccuracy(Abernethy*etal*.,2012;AdoneandPasquali,2013).Theserologicaltestsarepresumptivediagnosisforbrucellosisinanimalsaswellashuman(OIE,2012).

Severalserologicaltestsareusedtoday,butmostcommonlyusedserologicaltestsarescreeningtests(e.g.,RBPT),monitoringorepidemiologicalsurveillancetests(e.g.,milkringtest),andcomplementaryorconfirmatorytests(complementfixationtest,ELISAs).Selectionofagiventestshouldtakeintoaccountthespeciesoforganismandthelocalregulations(Nielsen,2002;Poester*etal*.,2010).Bodyfluidssuchasserum,uterinedischarge,vaginalmucus,milk,andsemenplasmafromsuspectedcattlemaycontaindifferentquantitiesofantibodiesoftheIgM,IgG1,IgG2andIgAtypesdirectedagainst*Brucella*(Zewdie,2018).

Milkringtest

Itischeap,easy,simpleandquicktoperform.Itdetectslactealanti*Brucella*IgMandfatglobulesfrommilkandformredringinpositivecase.However,ittestsfalsepositivewhenmilkthatcontainscolostrum,milkattheendofthelactationperiod,milkfromcowssufferingfromabnormaldisorderormastitis.MilkthatcontainlowconcentrationoflactealIgM,IgAorlackthefatclusteringfactors,te

stsfalsenegative.Becauselactealantibodiesrapidlydeclineafterabortionorparturition,thereliability ofmilkringtestusing1mlmilktodetect*Brucella*antibodiesinindividualcattleorintactmilkis stronglyreduced(Nielson*etal*.,2001).Althoughthemilkringtestperformedwith8mlmilk,itimproved thedetectionofbrucellosisintankmilk.Itmaytestfalsepositivewhenracesofcolostrumarepresentintankmilk(OIE,2009).

Rosebengalplatetest(RBPT)

TheRBPTisasimplespotagglutinationtestwheredropsofstainedantigenandserumaremixedonaplateandanyresultingagglutinationsignifiesapositivereaction.Itdoesneedspeciallaboratoryfacilitiesandiseasytoperform.Itusedtoscreenserafor*Brucella*antibodies.Thetestisanexcellentscreeningtestbutmaybeoversensitivefordiagnosisinindividualanimals,particularlyvaccinatedones(Munoz*etal.*,2005).AlthoughthelowPH(3.6)oftheantigenenhancesthespecificityofthetest,theambienttemperatureatwhichthereactiontakesplacemayinfluencethesensitivityandspecificityofthetest(Bricker,2002).

Complementfixationtest(CFT)

Complementfixationtest(CFT)isanothercommonlyusedserologicalmethods.Itisthemostreliablediagnostictestnowinroutineuseforindividualanimalsalthoughitiscomplextoperform,requiringgoodlaboratoryfacilitiesandadequatelytrainedstafftoaccuratelytitrateandmaintainthereagents.Itmeasuresmoreantibodiesoftheigg1typethanantibodiesoftheigmtype.Itisrelativelyinsensitivetoantibodyresultingfromstrain19immunizations(vaccinations).Therearenumerousvariationsofthecftinuse,butthistestismostconvenientlycarriedoutinamicrotiterformat(Nielson*etal*.,2001).Eitherwarmorcoldfixationmaybeusedfortheincubationofserum,antigenandcomplementateither37^{0}Cfor30minutesor4^{0}Cfor14–18hours.Anumberoffactorsaffectthechoiceofthemethod;anti-complementaryactivityinserumsamplesofpoorqualityismoreevidentwithcoldfixation,whilefixationat37°Cincreasesthefrequencyandintensityofprozonesandanumberofdilutionsmustbetestedforeachsample(Xavler*etal.*,2009).

Enzymelinkedimmunosorbentassay

Enzymelinkedimmunosorbentassay(ELISA)hasbecomepopularasastandardassayforthediagnosisofbrucellosisserologically.ItmeasuresIgG,IgAandIgMantibodiesandthisallowsabetteri

nterpretationoftheclinicalsituation.ThediagnosisofbrucellosisisbasedonthedetectionofantibodiesagainstthesmoothLPS.DetectionofIgGantibodiesismoresensitivethandetectionofIgMantibodiesfordiagnosingcasesofbrucellosisbutspecificityiscomparable(Araj,2010;Sathyanarayan*etal*.,2011;Agasthya*etal*.,2012).

TheindirectELISA(I-ELISA)hasbeenusedforserologicdiagnosisofbrucellosisinsheep,goatsandpigs.Ithasalsobeenusedfordiagnosisusingserumormilkfromcattle(DiFebo*etal*.,2012).I-ELISAhasbeenusuallyusedforsmoothLPS*Brucella*speciesanditissensitiveandspecificfor*B.abortus*or*B.melitensis*,butitisnotcapableofdifferentiatingantibodiesinducedbythevaccinestrainsS19orRev1(Lim*etal*.,2004;KhanandZahoor,2018).SensitivityofI-ELISAvariesfrom96to100%anditsspecificityfrom93.8%and100%(Gall&Nielsen,2004).

Ontheotherhandcompetitiveenzymelinkedimmunesorbentassays(C-ELISA)weredevelopedinordertoeliminatesome,butnotalloftheproblemsarisingfromresidualvaccinalantibody,andfromcross-reactingantibodies.Theassaysarecarriedoutbyselectingamonoclonalantibodywithslightlyhigheraffinityfortheantigenthanmostofthevaccinalorcross-reactingantibody,butwithloweraffinitythanantibodyarisingfrominfection(Munoz*etal*.,2005;OIE,2009;Poiester*etal*.,2010).Thespecificityofthecompetitiveenzymeimmunoassayisveryhighandisabletodetectallantibodyisotypes(IgM,IgG1,andIgG2andIgA)(Nielsen,2002).However,itisslightlylesssensitivethantheindirectenzymeimmunoassay.Thisassayisanoutstandingconfirmatoryassayforthediagnosisofbrucellosisinmostmammalianspecies.

Fluorescencepolarizationassay(FPA)

Itisbasedonthephysicalprincipleofthemass-dependentchangeofthemoleculesrotationspeedinaliquidmedium.Thesmallerthemolecule,thefasteritrotatesandthedepolarizationofapolarizedbeamoflightoccurs.InFPAtheserumsampleisincubatedwithaspecific*Brucella*antigen,conjugatedwithafluorescentlabel.Incasethereareanti-*Brucella*antibodiesintheserum,largefluorescentlylabeledantigen-antibodycomplexisformed,whichcaneasilybedistinguishedfromtheunboundantigennegativecontrol.FPAmethodhasahighspecificitybutlesssensitivitythanI-ELISA(McGiven*etal*.,2003).InEuropeandtheUSAFPAmethodisusedinprogramstomonitorandcontrolthespreadofbrucellosis,butitrequiresspecialequipmentanditisnotsuitableforrapidan

deasytesting.

2.7.3.Molecularmethods

Moleculartechniquesareimportanttoolsfordiagnosisandepidemiologicstudies,providingrele vantinformationforidentificationofspeciesandbiotypesof*Brucella*speciesallowingdifferentia tionbetweenvirulentandvaccinestrains(Queipo-Ortuño*etal.*,2008).Moleculardetectionof*Brucella*speciescanbedonedirectlyonclinicalsampl eswithoutpreviousisolationoftheorganism.Inaddition,thesetechniquescanbeusedtocomplem entresultsobtainedfromphenotypictests(Bricker,2002).DespitethehighdegreeofDNAhomolo gywithinthegenus*Brucella*,severalmolecularmethods,includingPCR,havebeendevelopedtha tallow,toacertainextent,differentiationbetween*Brucella*speciesandsomeoftheirbiovars(OIE, 2009;Colmenero*etal*.,2010).

Polymerasechainreaction

Thepolymerasechainreaction(PCR)isarecentandpromisingtechniquethatallowsaccuratediag nosisofbovinebrucellosis(Baddour,2012).Thetechniqueischosenbasedonthetypeofbiologica lsampleandthegoal,i.e.,diagnosisormolecularcharacterizationorepidemiologicalsurvey.Mos tofthemoleculardiagnosticmethodsforbrucellosishavesensitivityrangingfrom50%to100%an dspecificitybetween60%and98%.TheDNAextractionprotocol,typeofclinicalsample,anddete ctionlimitsofeachprotocol,arefactorsthatcaninfluencetheefficiencyofthetechnique(Mitika*et al*.,2007).

2.8.SignificanceoftheDisease

2.8.1.Economicsignificance

Endemicbrucellosisinlow-incomecountriesofsub-SaharanAfricaandSouthAsiahasmultipleeconomicimplicationsacrossagricultureandpublich ealthandbroadersocio-economicdevelopmentsectors.Effortstocontrolthediseaseinlow-incomecountriesmusttakeadifferentapproach.Simplyreplicatingpastsuccessesinbrucellosisc ontrolanderadicationinhigh-incomecountrieswillnotwork.Low-incomecountrieshaveatleastaten-foldhigherburdenofinfectiousdiseasefromawidevarietyofpathogens(McDermottandGrace,2 013).

Theassessmentoftheeconomicaspectsofbrucellosis,withemphasisonthelow-incomecountriesofAfricaandAsia,isstructuredinthreemainparts.Thefirstdescribesanoverallframeworkforeconomicassessmentofdiseaseburdensandtheimpactsofpotentialcontrolprograms.Thesecondpartsystematicallyreviewsavailableanimal,humanandjointburdenestimatesfromstudiesconductedintheseregions.Thethirdsectionprovidesestimates,whenavailable,ofdifferentcostsassociatedwithbrucellosisillnessanditscontrol.Thissectionalsocommentsontoolsandapproachesforassessingcontrolprogramsthatareofrelevancetolowandmiddle-income(Zamri-saadandKamarudin,2016).

Whenbrucellosisisdetectedinaherd,flock,region,orcountry,internationalveterinaryregulationsimposerestrictionsonanimalmovementsandtrade,whichresultinhugeeconomiclosses.Theeconomiclossesaswellasitszoonoticimportancearethereasonswhyprogramstocontroloreradicatebrucellosisincattle(OIE,2008).

InEthiopia,informationonlossesspecificallythroughbrucellosisinthedifferenttypesofproductionsystemsissparse,exceptforSintaro(1994)whoreportedanannuallossfrombrucellosisestimatedtobe88,941.96EthiopianBirr($5231equivalent)among193cattle,largelyduetoreducedmilkproductionandabortions(ChaffaStateFarm,Wollo,from1987to1993).

2.8.2.Publichealthsignificance

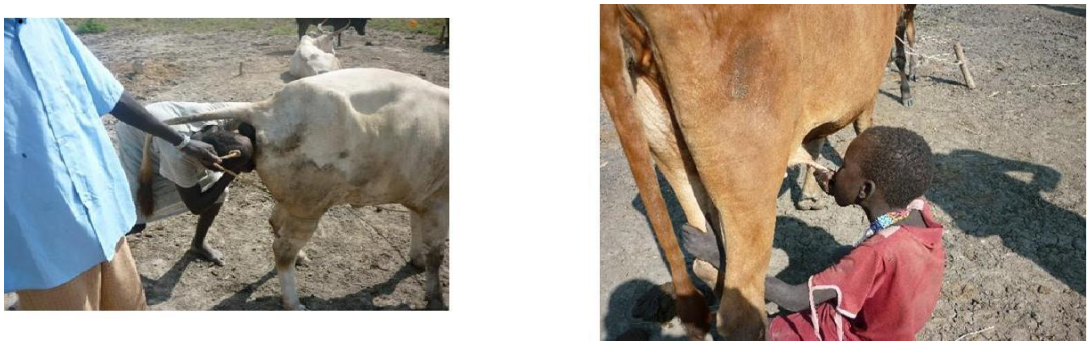
*Brucellaabortus,B.melitensis*and*B.suis*arehighlypathogenicforhumans(OIE,2009).Themajorityofreportedhumanbrucellosiscasesarecausedby*B.melitensis,B.abortus,*and*B.suis,*inoccurrenceorder,novelandatypical*Brucella*arealsobeinginvestigated(AlDahouk*etal.*,2013).Brucellosisremainsthemostcommonzoonoticdiseaseintheworld,withmorethan500,000newcasesreportedannually(Godfroid*etal.*,2013);theactualnumberofcases,includingundetectedandunreportedcases,isbelievedtobeconsiderablyhigher(AlDahouk*etal.*,2013).Brucellosisisoftenaneglecteddiseasedespitebeingendemicwithhighzoonoticpotentialinmanycountries(Poester*etal.*,2013).Theprevalenceofhumanbrucellosisdiffersbetweenareasandhasbeenreportedtovarywithstandardsofpersonalandenvironmentalhygiene,animalhusbandrypractices,andspeciesofthecausativeagentandlocalmethodsoffoodprocessing(Chugh,2008).

Ascomparedtostudyofanimalbrucellosis,studyofhumanbrucellosisinEthiopiaissparsewithevenlessinformationonriskfactorsforhumaninfection.Forinstance,outof56caseswithfeverofunknownorigin,two(3.6%)werereportedtobepositivefor*B.abortus*antibodiesbyRBPTandCFT(Jergafa*etal.*,2009).Astudyconductedintraditionalpastoralcommunitiesby Ragassa*etal.*(2009)r

evealedthat34.1%patientswithfebrileillnessfromBorena,29.4%patientsfromHammer,and3%patientsfromMetemaareasweretestedpositiveusing*Brucella*IgM/IgGlateralflowassay.Studiesconductedinhighriskgroupsuchasfarmers,veterinaryprofessionals,meatinspectorsandartificialinseminationtechniciansinAmharaRegionalState(Mussie,2007),SidamaZoneofSouthernPeopleNationsandNationalitiesSate(Kassahun*etal.*,2007),andAddisAbaba(Kassahun*etal.*,2006)foundaseroprevalenceof5.30%,3.78%and4.8%byscreeningserafrom238,38and336individualsrespectively.Thediscrepancybetweenandothersmightbeduetodifferenceinmilkconsumptionhabitsandsensitivityoftestmethodsused(Ferede*etal.*,2011).

Humansmaybecomeinfectedbyingestionofraworunpasteurizeddairyproducts,bydirecttransmissionthroughcontactwithinfectedanimalsorbyhandlingspecimenscontaining*Brucella*speciesinlaboratory.Italsotransmittedtohumanbydirectcontactwiththeskinormucosaduringparturitionandabortion(Degefu*etal.*,2011;Ferede*etal.*,2011;Addis,2015).

InSouthSudanafraughtwithseveralpotentialriskfactorscouldfuelthedisseminationofbrucellosistolivestockandhumans(Lado*etal.*,2012).Thetraditionalpastoralist'spracticeofassemblingseveralherdsintocattlecampswithcloselivestock-humaninteractionsisoneofthekeymilestones.Moreover,poorawarenessisariskmilestonetooccurrenceandperpetuationofbrucellosisinlivestockwhichcouldcreatehumanhealthhazards(Yohannes,2017).Furtherbrucellosisriskindicatorsincludingthewidespreadanimalherder'spracticeofvulvalblowing,tofacilitatemilklet-downduringcowmilking(figure4a)andthepracticeofdirectudder-to-mouthconsumptionofrawmilk(figure4b)couldexacerbatehumanbrucellosis(Lado*etal.*,2012)



(a)(b)

Figure4:Waysofdiseasetransmission(a)Blowingthroughthevulvatoenhancemilklet-down(b)DirectsucklingofrawmilkfromcattlecampsintheTerekekacountry.

Source:Lado*etal*.,2012.

2.9.Treatment

Aneffectivetreatmentforanimalswithbrucellosisisnotknowntodate(Tolosa,2004).Thetreatmentofbrucellosisinthecowhasgenerallybeenunsuccessfulbecauseoftheintracellularsequestrationoftheorganismsinlymphnodes,mammarygland,andreproductiveorgansandthebacteriaarefacultativeintracellularwhichsurviveandmultiplywithinthecells(Radostits*etal*.,2000).Generally,treatmentofinfectedlivestockisnotattemptedbecauseofthehightreatmentfailurerate,cost,andpotentialproblemsrelatedtomaintaininginfectedanimalsinthefaceofon-goingeradicationprograms(Asmre*etal*.,2010).Mancanbetreatedwithantibiotics(doxicyclinwithrifampicin);however,relapsesareimpossible(SmitsandKadri,2005).

2.10.PreventionandControl

Prevention,controlanderadicationofbrucellosisareamajorchallengeforpublichealthprograms.Althoughcontrolledoreradicatedinanimalsinanumberofdevelopedcountriesthroughacombinationofmassvaccination,testandslaughterprograms,effectivediseasesurveillanceandanimalmovementcontrolwhilethediseaseinhumanshasmajorlybeencontrolledthroughmilkpasteurization(McDermottandArimi,2002;Pappas*etal*.,2006),re-introductionofbrucellosisremainsaconstantthreat,whileinothers,especiallyinthedevelopingworld,thisdiseasecontinuestoexertitsdevastatingimpactperpetuatingpoverty(Smits*etal*.,2004).

AveryimportantapproachtothecontrolofbrucellosisthatisgainingmoreandmorerecognitioninrecentyearsistheOneHealthApproachtocontrolandpreventhumanandanimalbrucellosisrequiresmultidisciplineapproachsinceneitherveterinarianalonenorphysicianalonecouldn’tperformallapproachesofcontrol.Soitrequiresparticipationofotherdisciplineandfarmersforeffectivecontrolespeciallyindevelopingcountrieswheremostpeoplearelivingclosertoanimals(Pieracci*et al.*,2016).

IntheOneHealthframeworkveterinary,medical,environmentalandalliedprofessionalsandexpertscollaboratetogetherwiththeaimofidentifyingpossibleriskfactorsforthisinfectionanddesignasuitableapproachtocombattingtheinfection.Unfortunately,inmanyunderdevelopedanddevelopingcountries,thiskindofcollaborationisnon-existentorweakwhichgivesroomforbrucellosistothriveuncheckedespeciallyinruralpopulations(BeruktayitandMersha,2016).

InEthiopiatherehavebeennationalprogramsproposedforpreventionandcontrolofbrucellosisthroughOneHealthApproach.However,atregionallevels,nostrategyisinplacetocontrolbrucellosis.Thisislargelyaresultoflackoffacilitiesandbudgettorunsuchaprogram(BeruktayitandMersha2016).Thesuccessfulpreventionofthisdisease,whichissodifficultincattleproductioninthetropics,requiresthat,asfaraspossible,allavailablestepstakentocombatit(Yohannes*etal.*,2013).

2.10.1.Classificationofendemicareasbasedonprevalence

Classificationofendemicareasbasedonprevalencewillenableinitiationofappropriatecontrolmethodsinendemicareas.Identificationoflowandhighprevalenceareaswillgreatlyfacilitatetheimplementationofappropriatecontrolprograms,andshouldideallybecombinedwithotherstrategieslikeaccuratelivestockcensusdataandalivestockidentificationsystem(eithersimpleearnotchesormoresophisticatedearlabelingsystem).Inareaswherethediseaseislessprevalent(livestockseroprevalenceoflessthan1%),cullpolicywithcompensationmayberecommended.Forareaswithhighandmoderateprevalence(>5%)underwell-organizedfarmingsystems,wemayrecommendtestandsegregationpolicybywhichanimalswithbrucellosiswillbeisolatedandproductsconsumedafterpasteurization(Yohannes*etal.*,2013).

2.10.2.Characterizationof*Brucella*Species

Genotypingandidentificationof*Brucella*speciesbasedonmolecularapproacheshaveprovedtobepowerfultoolstoconfirmthediseaseandtoidentify*Brucella*speciesandits*biovars*and*Brucella*likeorganisms.Asaprerequisite,*Brucella*speciesidentificationshouldbeundertakentoinformselectionofthemostappropriatevaccine(forexample,*Bmelitensis*hasrecentlybeenfoundinfectingcattleinKenya)andtoenabledifferentiationofvaccineandwild-typestrains(Muendo*etal.*,2012).

2.10.3.Vaccination

TheWHOhaslongbeeninvolvedinbrucellosissurveillanceandcontrol,includingresearchanddevelopmentofvaccinestopreventanimalbrucellosis(Munir*etal.*,2010).Systematicvaccinationofanimalsisrecommendedwheretheprevalenceisgreaterthan5%(Holvoet*etal.*,2007).Vaccineincreasesindividualresistancetosystemicinfection,andininfectedanimalsdecreasestheprobabilityofplacentalinfection,abortionandmassivesheddingofinfectiousorganisms(Ibrahim,2010).Indifferentpartsoftheworldbothlivevaccines,suchas*B.abortus*S-19,*B.melitensis*Rev-1,*B.suis*S-2,rough*B.melitensis*strainM111,and*B.abortus*strainRB-

51andkilledvaccines,suchas*B.abortus*45/20and*Bmelitensis*H-38areavailable.Eachvaccinehasbeenreportedtohaveitsownadvantagesanddisadvantages,withprotectionfollowinglocalizedpersistenceoflivevaccinespreferredbymostandshowingefficacyinsmallruminantsandcattle(ThakurandThapliyal,2002).

BrucellaabortusS19Vaccine

Themostwidelyusedvaccineforthepreventionofbrucellosisincattleisthe*Brucellaabortus*S19vaccine,whichremainsthereferencevaccinetowhichanyothervaccinesarecompared.Itisusedasalivevaccineandisnormallygiventofemalecalvesagedbetween3and6monthsasasinglesubcutaneousdoseof5–8×10^{10}viableorganisms.Areduceddoseoforganismscanbeadministeredsubcutaneouslytoadultcattle,butsomeanimalsmayabortandexcretethevaccinestraininthemilk.Alternatively,itcanbeadministeredtocattleofanyageseitheroneortwodosesof5×10^{10}viableorganisms,givenbytheconjunctivalroute;thisproducesprotectionwithouttherisksofabortionandexcretioninmilkwhenvaccinatingadultcattle(Seleem*etal.*,2010).*Brucellaabortus*S19vaccineinducesgoodimmunitytomoderatechallengebyvirulentorganisms.SeedlotsforS19vaccineproductionshouldberegularlytestedforresidualvirulenceandimmunogenicityinmice(Seleem*etal.*,2010).

BrucellaabortusstrainRB51vaccines

Thisisarecentlydevelopedvaccineandhasreplaced*Br.abortus*strain19inanumberofcountriesastheapprovedcalfhoodvaccinebecauseitdoesnotinterferewithserologicalevaluation(Asmare*etal.*,2010).*Brucellaabortus*strainRB51isalivestableroughmutantof*Br.abortus*strain2308,whichlacksmuchofthelipopolysaccharideO-sidechainandhasbeeninvestigatedasanalternativetostrain19vaccines(Radostits*etal.*,2000).Adultvaccinationswith*Br.abortus*strainRB51onlyrarelycausesabortion.Onewaytoreducethesideeffectsof RB51istoreducethedose.Whenusingthereduceddoseofthisvaccine(1×10^{10}colony-formingunits[CFU]),onlatepregnantcattle,noabortionsorplacentitislesionsareproduced(DinkaandChala,2009).

2.10.4.Applicationofveterinaryextension

Healtheducationisanotheroptiontoreduceoccupationalandfood-bornerisks.Theultimatepreventionofhumaninfectionremainstheeliminationofinfectionamon

ganimals(Radostits*etal*.,2000).Thedevelopmentofanationalveterinaryextensionservicesinth ecountry,iscriticaltopromoteawarenessaboutbrucellosis,itsimpactonlivestockproductionand zoonoticrisks.Everybodyhasresponsibilitytokeephisenvironment,animalsandownhealthcare .Toloweryourriskofgettingbrucellosisfromnaturalsource;avoideatingordrinkingunpasteuriz edmilk,cheeseoricecream;checkthelabeltomakesureitsays“pasteurized”anddon’teatitifyouar enotsure;donothandlesickordeadanimalbodies,butifyoumust,thenuseglovesandprotectivem aterials;cookmeatthoroughlyanddisinfectingtheareawheretheanimalsareaborted(Beruktayit *etal*.,2016).

3.MATERIALANDMETHODS

3.1.StudyArea

Thestudywascarriedoutinthreepurposelyselecteddistrictsof EastWollegazonenamelyGobuS eyo,SibuSireandGudeyaBilaintheperiodfromNovember2018toSeptember2019.GobuSeyodi strictissituatedinEastWollegazone265kmwestofAddisAbababorderedbyWestShewazoneint heeast,SibuSireinthewest,GudeyaBilainthenorthandBonayaBosheinthesouth.Thecapitaltow

nofGobuSeyo(Ano)islocated65kmtoeastfromNekemte,thecapitaltownofEastWollegazone. Thedistricthasanaltituderangingfrom1300-2998meterabovesealevelandtemperatureofthearearangesfrom13.6^{0}Cto28.8^{0}C.Thisdistrictha s76,791ofCattle,5,334ofSheep,9253ofGoat,720ofHorses,601ofMules,3300ofDonkeys(GS WOARD,2017).

SibuSireisoneofthedistrictsoftheEastWollegazonewhichborderedonthesouthbyWamaBona ya,onthewestbyGutoWayu,andonthenorthandeastbyGobuSeyoandGudeyaBiladistrictsofEa stWollega.Theadministrativecenterofthisdistrictis Sire.SibuSiredistrictislocatedabout272km westofthecapitalcityofEthiopia,AddisAbaba.Itliesbetween8°56'-9°23'Nlatitudesand36°35'-36°56'Elongitudes.Thealtitudeofthedistrictvariesfrom1336to2500meterabovesealevel.Abo ut74.2%ofitssurfaceareabelongstomid-altitudeagro-climate,7.53%ofthelandishighlandagro-climateandtheremaining18.27%isclassifiedaslowlandagro-climate.Themeanannualtemperatureandmeanannualrainfallis25^{0}Cand1050mm,respectively .Thelivestockpopulationoftheareais(Cattle125,343,Sheep14,502,Goat24,212,Horse5685,M ule1023,Donkey8415and57,695poultry(SSWOARD,2017).

GudeyaBilaisoneofthedistrictsintheEastWollegazoneanditwaspartofformerBilaSeyoworeda .Itislocatedatthedistanceof270KmfromAddisAbabatowest.ThedistrictisborderedbyJimaGen etidistrictofHoroGuduruwollegaineast,BakoTibedistrictofWestShoainsouth-east,GobuSayoandSibuSiredistrictsofEastWollegainwestandAbeDongorodistrictofHoroGu duruWollegainnorth.Thereare13peasantassociationsand2townadministrations(BilaandJare) .TheareahasrainfallfromJunetoSeptemberandadryseasonfromOctobertoMay,withameanann ualrainfallof1100mm-1950mm.Thealtituderangesfrom1100m–2400m.Thedailyaverageminimumandmaximumtemperaturesare18.5^{0}Cand27.5^{0}C,respectiv ely.AccordingtotheWoreda'sOfficeofAgricultureandRuralDevelopment,theanimalpopulati onsofthestudyareain2017were104,567cattle,85,743sheep,106,212goats,63,685horses,2482 donkeysand1632mules(GBWOARD,2017).

Mixedcrop-livestock,extensivesystemisthemainproductionsystempracticedinthearea.Almostalltypesofl ivestockspeciesarebeingrearedinthestudyzone.However,cattlearethepredominantinthearea. Cattleareusedasassetsandaretheonlysourceoftractionpowerbesidesmilkandmeat

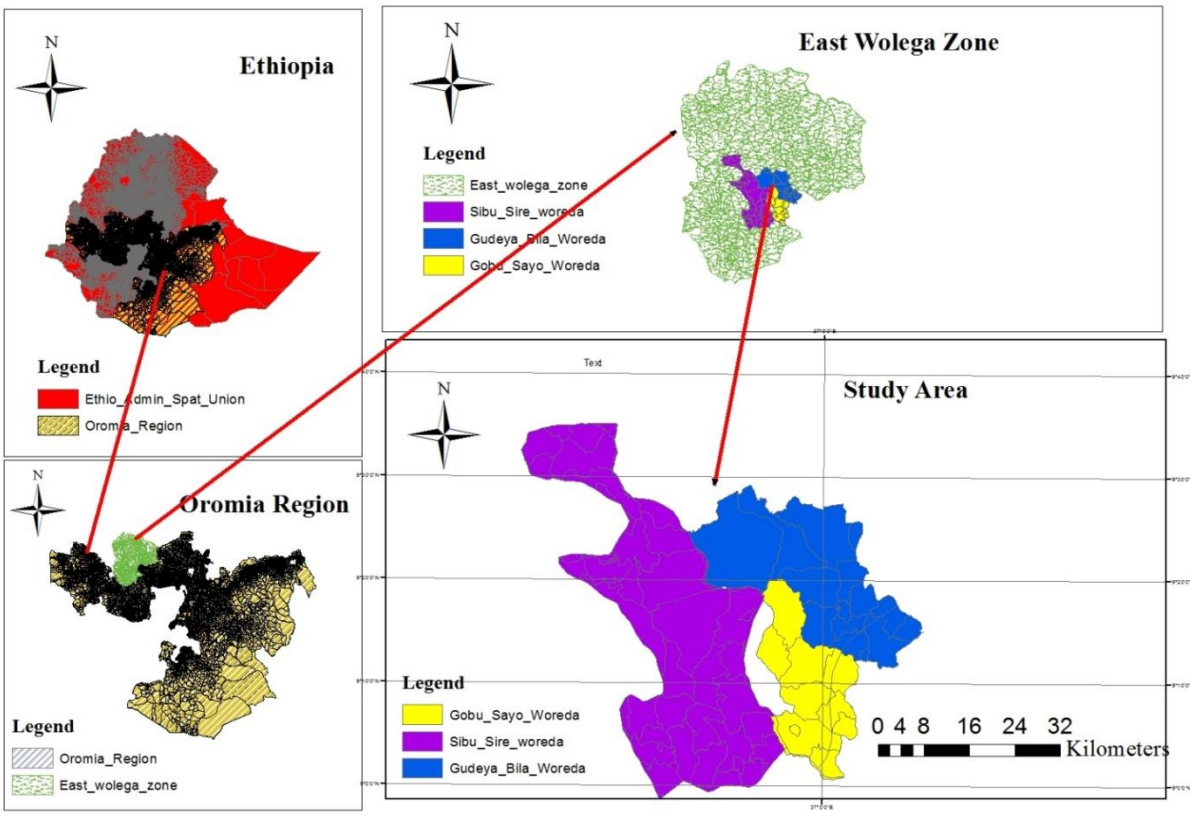


Figure5:Mapofstudyarea

3.2.StudyAnimalsandtheirManagement

Thestudypopulationusedinthisstudywereallcattlepopulationabove6monthsofagewithnohistoryofvaccinationagainstbrucellosisinselecteddistrictsofEastWollegazonethatwerekeptunderextensiveandsemi-intensivemanagementsystem.Classificationofmanagementsystemswasdonebasedonthecriteriaadoptedby Rashid(1993).Bothsexesanddifferentagegroupgreaterthansixmonthwereincludedinthestudyasthediseaseisnotcommoninthecattlelessthan6monthsofageduetomaternalantibody.

3.3.StudyDesign

Across-sectionalstudywascarriedoutinindigenousbreedsofcattleunderextensivehusbandryandcrossbreeds(ZebuwithHolsteinFriesian)ofcattleundersemi-intensivehusbandrytoassessseroprevalanceofbovinebrucellosisandtheirassociationwithdifferentriskfactors.Semi-structuredquestionnairesurveywasalsoconductedtocollectdataonfactorsexpectedtobeassocia

tedwithepidemiologyandtransmissionofbrucellosis.

3.4.SamplingProcedureandSampleSizeDetermination

Thestudyzoneanddistrictswereselectedpurposively.Peasantassociations(PA's)weretakenrandomlyaccordingtotheproportionsofPA'sfoundineachdistrict.Accordingly,2PA'sfromGobuSeyo,3PA'sfromGudeyaBilaand4PA'sfromSibuSiredistrictswereincludedinsampling.Ninepeasantassociationsweresampledfromatotalof43(GobuSeyo:9,SibuSire:19,GudeyaBila:15)inthesethreedistricts.Thiswasfollowedbysamplingofherds(households)intheselectedpeasantassociation.Thosecattlethathousedinthesamebarnsorunderindividualhouseholdswereconsideredasoneherd(Tolosa,2004;Asgedom*etal.*,2016).Accordingtodataobtainedfromthedistrictagriculturaloffice,thenumberofhouseholdsineachPA'svariesfrom350to400.Ineachstudyarea,thelistsofhouseholddataweregainedfromthekebelemanager.Averagesof8herds(households)wereselectedbysystematicrandomsamplingmethodfromeachPA.Atotalof75herds(20fromGobuSeyo,32fromSibuSireand23fromGudeyaBila)wereincludedinsampling.Animalsabovesixmonthsofagewithintheherdswereselectedusingsimplerandomsamplingmethod.

Additionallyallfarmsofmainlycrossbreedcattleweresampledpurposively,sincetherewerefewcrossbreedcattleinstudyarea.Farmsweredividedintothreecategories;smallscale(≤10headsofcattle),mediumscale(≥10-20headsofcattle)andlargescale(≥20headsofcattle)dependingonnumberofanimals(Boyazoglu,1998).Totally12farms(twolargescalefarm,fivemediumscalefarmsandfivesmallscalefarm)wereincludedinsampling.Studyanimalswereselectedbysimplerandomsamplingmethod(lotterymethoddependingontheireartag,nameandcolor).Thenumbersofanimalssampledfromeachscaleweredeterminedbytheproportionofthecattlepopulationexistingineachscale.

Inordertodeterminethedesiredsamplesize,therewerenopreviousreportsofbovinebrucellosisprevalenceinthepresentstudyarea.Therefore,theaverageexpectedprevalenceratewasassumedtobe50%fortheareawithin95%confidenceinterval(CI)at5%desiredprecisionasstatedbyThrusfield(2007).Hence,usingtheformula,calculatedsampleforthecurrentstudybecomes384headsofcattle;however,atotalof488serumsamples(362fromlocalbreedand126fromcrossbreed)ofbothsexesweresampledfrom87herdsinthestudyareastoincreasetheprecisionoftheresult.

$$n = \frac{Z^2 \times p_{expe}(1-p_{expe})}{d^2}$$

Where,n=requiredsamplesize

Pexp=expectedprevalence

d=desiredabsoluteprecision

Z=confidencestatistics

Table2.Thenumberofanimalssampledfromeachdistricts

District	Numberofanimalsampled
GobuSeyo	149
SibuSire	188
GudayaBila	151
Total	488

3.5.SampleandDataCollection

3.5.1.Bloodsamplecollection

Animalswererestrainedbyanimalhandlersandapproximately10mlofbloodsamplewascollected fromthejugularveinafterdisinfectionofthesiteusingvacutainertubeswith18-20gaugehypodermicneedles.Eachbloodsamplefromeachanimalwaslabeledonvacutainertube byusingcodesdescribingthespecificanimalandkeptovernightatroomtemperaturetoallowclotting.Atthenextmorningclearlyseparatedserumofapproximately2mlweredecantedtothecryo-vialstowhichidentificationwascoincided.Theobtainedserawerestoredat-20^0CuntiltestedbybothRoseBengalPlateTestandC-ELISAtestmaterials.Duringbloodsampling,epidemiologicaldataforstudyatindividualanimallevelwerecollectedusingsampledatacollectionsheet(Annex1).

3.5.2.Questionnairesurvey

Verbalagreementwasobtainedfromtherespondentsandtheobjectiveofthesurveyexplainedtothembeforestartingtheinterview.A12semi-intensiveand75extensive,totalof87ownersorrespondentswereinterviewedinlocallanguages(AfaanOromo)paralleltobloodcollectionusingsemi-structuredquestionnairethatwere believed to influence the epidemiology and transmission of Brucellosis(Annex2).Similarly,informationrelatedtotheanimalsattributeslikebreed,sex,age,reproductivestatus,parity,originoftheanimal,historyofabortionandretainedfatalmembranewer

ecollected.Basedonitsbiologicalrelevance,agewasstratifiedintothreecategories:<3years,≥3–6yearsand>6yearsaccordingtodentaleruption(PaceandWakeman,2003)(Annex3).Besides,informationonfarmssuchas:herdsize,managementsystems,matingmethod,presenceofparturitionpen,disposalafterbirthandotherriskfactorswerealsocollectedusingaquestionnaireformatpreparedforthispurpose.

3.6.SerologicalTests

3.6.1.Rosebengalplatetest(RBPT)

AllserumsampleswerescreenedusingtheRBPTatBedeleVeterinaryRegionallaboratoryaccordingtoOIE(2016)procedures(Annex4).Seraandantigenweretakenfromrefrigeratorandleftatroomtemperatureforhalfanhourbeforethetestconducted.Briefly,30µlofeachtestserumweretakenandplacedonacleanglassslide.Thenthesameamountsof30µlofRBPTantigenwereaddedtothesideofeachtestserum.Theantigenandtestserumweremixedthoroughlyinaplasticapplicator,shakenfor4 min,andagglutinationwasreadimmediately.Anyobservedagglutinationbythenakedeyewasconsideredtobeapositivereaction.

3.6.2.CompetitiveELISA

AllRBPTpositiveserawerefurthertestedusingtheCOMPELISA160and400,acompetitiveELISAkitforthedetectionofantibodiesagainst*Brucella*inserumsamples(NewHaw,Addlestone,Surrey,KT153NB,UnitedKingdom)attheNationalVeterinaryInstitute(NVI),Bishoftu,Ethiopia.Thetestwasperformedaccordingtothemanufacturer'smanualin96-wellpolystyreneplatesthatwerepre-coatedwith*Brucella*speciesLipopolysaccharide(LPS)antigen(Annex5).20µlofeachtestserumwasaddedtoeachwellfollowedby100µlofpreparedconjugatesolution.Theplateswerethenshakenvigorouslyfortwominutesandincubatedatroomtemperaturefor30 minonrotaryshaker,at160 revs/min.Plateswerewashed5timesanddried.100µlofP-PhenylePhosphatesolutionwasaddedtoallwellsandtheplateswereincubatedatroomtemperaturefor10to20 min.Thereactionwasthenbeingstoppedusingstoppingsolution.Opticaldensities(OD)werereadat450 nmusingmicroplatereader.Thelackofcolordevelopmentindicatedthatthesampletestedwaspositive.Apositive/negativecut-offwascalculatedas60%ofthemeanoftheODofthe4conjugatecontrolwells.Anytestsamplegivinganodbelowthisvaluewastakenasbeingpositive.

3.7.DataManagementandAnalysis

Descriptivestatisticwasutilizedtosummarizedataaftercodedandtransferredto StatisticalPackageforthe SocialScience(SPSS)version20.Twoepidemiologicalparameterswereregeneratednamelyindividualanimalandherdlevelseroprevalence.Individualanimalseroprevalencewascalculatedbythenumberofpositiveanimalsdividedbythetotalnumberofanimalstested.Similarly,herdlevelprevalencewascalculatedbythenumberofpositiveherdswithatleastoneseropositiveanimalintheherddividedbythetotalnumberofherdsscreened.Ananimalwasconsideredpositiveifittestedseropositiveon C-ELISAtest.Univariablelogisticregressionanalysiswasusedtoselecttheindividualexplanatoryvariablethatmaypredicttheoutcomevariableinthemodel.Theexplanatoryvariables(P≤0.25)werefurthercheckedformulticollinearityusingthevarianceinflationfactor(VIF)andtolerancefactor(TF)beforemultivariablelogisticregressionanalysis.Varianceinflationfactorvaluesofgreaterthan3ortolerancelessthan0.1wereconsideredthecut-offpointsforthecollinearitydiagnostics.Thestrengthofassociationbetweenoutcome(*Brucella* seropositivity)andriskfactorswasassedusingtheoddratio(OR).Multivariablelogisticregression analysiswasconductedtocalculatetheprobabilityofdiseasehappeningasafunctionofseveralindependentvariables.Thebackwardeliminationprocedurewasusedtoeliminatethefactorsthatwerenotsignificantat P<0.05inoverallmodel.Factorsthatweresignificant(P≤0.05)wereretainedinthefinalmodelandmodelfitwasobservedusingtheHosmer-Lemeshowtest.

4.RESULTS

4.1.Overallseroprevalenceofbovinebrucellosis

Inthepresentstudy,atotalof488cattle(157male(32.17%)and331female(67.83%))serawerecollectedfromanimalsabovesixmonthsofagewhichwerenotvaccinatedagainstbovinebrucellosis. Ofthem,11(2.25%)(95%CI:0.94-3.5)werepositiveinaRBPTtestandsixwereconfirmedtobeseropositiveforbrucellosisusingC-ELISA,givingseroprevalenceof1.23%(95%CI:0.25-2.2).Outof87herdsincludedinthestudy,6(6.9%)(95%CI:3.2-14.2)wereseropositiveusingC-ELISAwithatleastoneseropositiveanimalintheherd.Anoverallanimallevelseroprevalenceof1.23%andherdlevelseroprevalenceof6.9%wererecorded(Table3).

Table3.Overallindividualanimalandherdlevelbrucellosisseroprevalencebasedon RBPTandELISAtest.

Testassay	Classification	Animallevel			Herdlevel		
		N	%	95%CI	NF	%	95%CI
RBPT	Negative	477			78		
	Positive	11	2.25	0.94-3.5	9	10.34	3.9-16.74
C-ELISA	Negative	482			81		
	Positive	6	1.23	0.25-2	6	6.9	3.2-14.2
Total		488			87		

N:numberofanimalsNF:numberoffarm%:Prevalence

4.2.Riskfactorsanalysis

4.2.1.Animallevelriskfactorsanalysis

Theresultsofanimallevel*Brucella*seropositivityandtheirassociationwithexposurevariablesusingunivariablelogisticregressionwerepresentedin Table4. Accordingly, seroprevalenceofbovinebrucellosiswasnotsignificantlyrelatedwithstudydistricts(P>0.05). Thoughtherewerenosignificantdifferenceamongstudydistrictsand*Brucella*seropositivity, slightlyhigherproportionofseropositivitywasobservedinGobuSeyodistrict(2%)whencomparedtoSibuSire(0.53%)andGudeyaBila(1.34%)districts. Sexwasfoundnotasignificantfactorforbrucellosisinfection,(P=0.428)despitefemaleshavingaslightlyhigherproportionofinfection1.5%(n=331)comparedtomales0.64%(n=157). Seroprevalenceofbovinebrucellosiswassignificantlyrelatedwithageofanimal(P<0.05). Seroprevalenceof5.37%wasobservedinolderanimals(>6years)and0.42%inanimals≥3-6yearsold. Noanimallessthan3yearsoldwasfoundtobeseroreactive. Among331femaleanimalstested15(4.5%)showedhistoryofabortion,28(8.45%)withhistoryofretainedplacenta,44(13.29%)werepregnantand287(87%)werenonpregnant(heifers,lactatinganddrycows). Theseroprevalencesofbrucellosiswerealsosignificantlyassociatedwithabortingcows(P=0.000),retentionofplacenta(P=0.047)andpregnancystatusofananimal(P=0.004). Thestudyfailstodetectasignificantvariationin*Brucella*seropositivitybetweenbreeds,animaloriginandparityatindividualanimal. AnimalswithzeroparitywerenegativeinbothRBPTandC-ELISA. Thoughnumberofparitieswasnotsignificantatthe5%level,sincetheirPvalueswere≤0.25theywereconsideredaspotentialriskfactorsandthussubjectedtothemultivariablelogisticregressionanalysis.

Table4.Univariablelogisticregressionanalysisofcommonriskfactorsassociatedwith*Brucella*seropositivityatindividualanimallevel

Riskfactors	N	C-ELISA Positive(%)	OR(95%CI)	p-value

Districts				
SibuSire	188	1(0.53%)		
GudeyaBila	151	2(1.34%)	0.12(0.014-1.05)	0.56
GobuSeyo	149	3(2%)	1.79(0.328-2.353)	0.796
Sex				
Male	157	1(0.64%)		
Female	331	5(1.5%)	2.39(0.277-20.65)	0.428
Age				
Young(<3years)	159	0		
Adult(≥3-6years)	236	1(0.42%)	4.25(2.65-46.35)	0.025
Old(>6years)	93	5(5.37%)	6.7(1.452-30.97)	0.015
Breed				
Cross	126	1(0.79%)		
Local	362	5(1.38%)	0.57(0.066-4.937)	0.611
Stockreplacement				
Purchased/bought	114	1(0.87%)		
Self-reared/born	374	5(1.33%)	0.65(0.076-5.648)	0.697
Parity				
Nullparous	132	0		
Monoparous	80	1(1.25%)	3.5(0.217-56.69)	0.183
Biparous	91	3(3.297%)	8.39(0.86-81.63)	0.067
Multiparous	28	1(3.58%)	10.25(0.624-68.6)	0.103
Pregnancystatus				
Nonpregnant	287	2(0.69%)		
Pregnant	44	3(6.8%)	16.09(2.84-23.89)	0.004
Historyofabortion				
Absent	316	2(0.64%)		
Present	15	3(20%)	16(9.27-14.48)	0.000
HistoryofRFM				
Absent	303	2(0.67%)		
Present	28	3(10.71%)	12.7(6.7-29.79)	0.047

N=numberofanimalsscreened

4.2.2.Herdlevelriskfactorsanalysis

Theherdlevelunivariablelogisticregressionanalysisrevealedthatherdsizeswerefoundtobestronglyassociatedwithherdseropositivityto*Brucella*infection(P<0.05).Therewasnosignificantdifferenceof*Brucella*seropositivityaccordingtomanagementsystems(P=0.902).Howeverrelativelyhigherproportionofseropositivitywasobservedinextensivemanagement(8.33%)whencomparedtosemiintensivemanagementsystem(6.66%).Thestudyalsofailstodetectasignificantvariationin*Brucella*seropositivityamongotherriskfactorsatherdlevel(Table5).

Table5.Univariablelogisticregressionanalysisofrisk/indicatorfactorsforherdlevelbrucellosis seropositivity

Riskfactors	NF	C-ELISA Positive(%)	OR(95%CI)	p-value
Districts				
SibuSire	36	1(2.77%)		

GudeyaBila	25	2(8%)	0.95(0.82-4.165)	0.45
GobuSeyo	26	3(11.53%)	0.6(0.215-1.748)	0.36
Managementsystem				
Semi-intensive	12	1(6.66%)		
Extensive	75	5(8.33%)	1.15(0.123-10.725)	0.902
HerdSize				
≤10	24	0		
≥10-20	29	2(6.8%)	3.45(1.87-27.65)	0.026
≥20	34	4(11.76%)	8.45(1.18-22.28)	0.011
MatingMethod				
Artificial	2	0		
Natural	79	5(6.32%)	1.63(0.498-5.374)	0.417
Both	6	1(16.66%)	2.62(0.612-8.643)	0.773
Parturitionpen				
Present	8	1(12.5%)		
Absent	79	5(6.32%)	5.2(0.45-5.94)	0.185
Disposalafterbirth				
Present	14	1(7.15%)		
Absent	73	5(6.8%)	1.05(0.113-9.705)	0.968

NF=numberoffarms

InTable6,theresultsofmultivariablelogisticregressionanalysisshowingimportantriskfactorsfor*Brucella*seropositivity.Riskfactorswithp-value≤0.25intheunivariablelogisticregressionmodelwhereincludedinthemultivariablelogisticregressionmodel.Accordingly;age,herdsize,parity,pregnancystatusoftheanimal,presenceofparturitionpen,historyofabortionandretainedfetalmembranewereincludedinthefinallogisticregressionmodel.However,inthefinalanalysisanimalsseropositivitywasinfluencedmorebyherdsize,age,pregnancystatusofanimalsandabortioncase.Thusmultivariablelogisticregressionanalysisdepictsthatbrucellosisseropositivitywerefoundtobe6.5(95%CI:1.459-28.967)timeshigheramongolderanimalsgreatersixyearsthanyoungeranimals.Animalsinvolvedinlargeherdsizewere8.5timesmorelikelytobeathigherriskfor*Brucella*infectionthananimalsinsmallherdsize(95%CI:1.217-19.872,P=0.031).Similarly,pregnancystatusinfemaleswerefoundtobesignificantlyassociatedwithseropositivity(P=0.009).Brucellosisseropositivitywasfoundtobe12.8(95%CI2.35-45.72)timeshigheramongpregnantanimalscomparedtothoseofthenon-pregnantanimals.Theseroprevalenceofbrucellosiswasalsosignificantlyhigherinfemaleanimalsthosehadahistoryofabortion(20%)comparedwithnohistoryofabortion(0.7%)(95%CI:6.759-10.389,OR=8.3,P=.0.001).Thiscouldbeexplainedbythefactthatabortionistypicaloutcomesof

brucellosis.Therestriskfactorshowednostatisticallysignificantassociationsregardlessoftheseropositivityrecorded.

Table6.Multivariablelogisticregressionanalysesidentifyingtheassociationofpotentialriskfactorsto*Brucella*seropositivityincattle

RiskFactors	Categories	95%CI	OR	p-value
Age	Young(<3years)*			
	Adult(≥3-6years)	2.265-15.653	3.15	
	Old(>6years)	1.459-28.967	6.5	0.014
HerdSize	Small(≤10headsofcattle)*			
	Medium(≥10-20headsofcattle)	1.05-5.654	2.45	0.046
	Large(≥20headsofcattle)	1.217-19.872	8.56	0.031
Pregnancystatus	Nonpregnant*			
	Pregnant	2.350-45.72	12.78	0.009
Historyofabortion	Absent*			
	Present	6.759-10.389	8.3	0.001

*:Referancecategory;CI:Confidenceinterval;OR:Oddratio

4.3.QuestionnaireSurvey

Themajorityofparticipants,97.35%ofextensivefarmownersorrespondentsand83.34%extensivefarmownersorrespondentswerenotawareofbovinebrucellosis.Respondentswerealsointerviewedtodescribetheoccurrenceofsomereproductiveproblemsandindicated12.65%abortion.Mostoftherespondents(85%)hadnoknowledgeoncausesofabortionandasbrucellosiscauseabortionincattleand96.55%ofthemhadnotisolatingabortedanimalfromothers.Thepracticesofproperlydisposingafterbirthweredonerelativelyinabetterwaybyfarmersinsemi-intensivemanagementsystem.Themajorityoftherespondentsconsumerawmilk(80.45%)andrawmeat(94.25%).Similarly,mostofthefarmers(87.35%)havehabitofassistingcowsduringparturition,ofwhichonlyveryfew(3.5%)ofthemuseprotectiveglove(Table7).

Table7**.**Knowledge-attitudesandpracticesoffarmownersaboutbrucellosis

Variable	Extensivefarm (n=75)	Semi-intensivefarm(n=12)	Total (n=87)
Awarenessaboutbrucellosis			
Yes	2(2.65%)	2(16.66%)	4(4.6%)
No	73(97.35%)	10(83.34%)	76(95.4%)
Abortion			
Yes	7(9.33%)	4(33.33)	11(12.65%)
No	68(90.66%)	8(66.67%)	76(87.34%)

Perceptiononcauseofabortion			
Yes	9(12%)	4(33.33%)	13(15%)
No	66(88	8(66.67%)	74(85%)
Separationofabortedcow			
Yes	1(1.33%)	2(16.66%)	3(3.45%)
No	74(98.67%)	10(83.37%)	84(96.55%)
Properdisposalofafterbirth			
Burial	5(6.7%)	6(50%)	11(12.64%)
Burning	2(2.7%)	2(15.38%)	4(4.6%)
Opendump	68(90.6%)	4(33.32%)	72(82.8%)
Rawmilkconsumption			
Yes	65(86.66%)	5(41.66%)	70(80.45%)
No	10(13.34%)	7(58.34%)	17(19.55%)
Rawmeatconsumption			
Yes	71(95.67%)	11(91.66%)	82(94.25%)
No	4(5.33%)	1(8.34%)	5(5.75%)
Assistingcowduringparturition			
Yes	68(90.66%)	8(66.66%)	76(87.35%)
No	7(9.34%)	4(33.34%)	11(12.64%)
Useprotectiveglovesduringassisting			
Yes	-	3(37.5%)	3(3.5%)
No	68(100%)	5(62.5%)	73(96.5%)

5.DISCUSSION

Thestudyrevealedthattheoverallprevalenceofbovinebrucellosiswas1.23%inthethreeselected districtsofEastWollegaZone.Theprevalenceinthisstudywassimilar to thefindingsofTefera(2006)withprevalenceof1.13%inintensiveandextensivefarmsofAddisA babaandSululta,Degefu*etal*.(2011)whofoundanoverallprevalenceof1.38%fromAgropastora lcattle'sofJijjiga,Berhe*etal.(*2007)whofoundanoverallprevalenceof1.49%inextensiveandse mi-
intensivefarmsofTigrayRegion,Asmare*etal*.(2010)withcattleprevalence1.92%inSidamaZon e,Yohannes*etal*.(2012)withcattleprevalenceof1.97%inGutoGidadistrictsofEastWollegazon e,Roba(2017)withprevalenceof1.1%inDidaTuyuraRanchandpastoralherdsofBorenazone,Y ohannes(2017)withprevalenceof1.3inHumbodistrictsofWolaitazone.

Thepresentstudywaslowerthanmanyoftheearlierreportsin Ethiopia.Forinstances,higherpreva

lencethanthecurrentreportwasobservedbyvariousauthors(4.63%inextensiveandintensivefarmsinBahirdarbyHailemelekot,2005,11%insmallholderfarmsinCentralEthiopia(Wuchale-Jidadistrict)byKebede*etal.*,2008,7.7%prevalenceofbovinebrucellosisinextensiveandsemi-intensivefarmsofTigrayregionbyHailessilasise*etal.*,2010,2.43%inJijigabyBekele*etal*.,2011,14.14%prevalenceofbovinebrucellosisinAsselagovernmentdairyfarmofOromiaregionalstatebyDeselgnandGangwar,2011,2.9%prevalenceinArsizoneofOromiaregionalstatebyTsegaye*etal*.,2016).

Tadese(2003)innorthGonder,Mussei(2007)incrossbreedsofBahirdar,Lidia(2008)incentralhighlandsofEthiopia,Degefa*etal.*(2011)inArsizoneofOromiaregionalstate,Tesfaye*etal*.(2011)indairyfarmsofAddisAbaba,Bashitu*etal*.(2015)incrossbreedsofDebrebirhanhavereportedprevalenceof0.14%,0.26%,0.45%,0.05%,0.69%,and0.2%respectivelywhichslightlyloweroverallprevalencewhencomparedtothisfinding.

Thedifferenceinprevalenceobservedbetweenthereportsfromdifferentpartsof Ethiopiaandthepresentstudymaybeduetodifferencesinherdsize,samplesize,testsused,agroecologicalandmanagementconditions,andthepresenceorabsenceofinfectiousfoci,suchas*Brucella*-infectedherds,whichcouldspreadthediseaseamongcontactherds.

Thepresentstudyshowedthattherewasnon-significantdifferenceinseroprevalenceofbrucellosisamongthreestudydistricts(GobuSeyo,SibuSireandGedeyaBila).Thiscouldbeduetosimilarityamongtraditionalmanagementsystem.

Thoughinthepresentstudytheseroprevalenceofbovinebrucellosiswasnotstatisticallysignificantbetweensexes,theresultshowedthatinfectionwashigherinfemale(1.42%)thanmale(0.63%). Thisfindingwasinagreementwiththefindingsof Hailemelekot*etal.*(2007)inTigrayregion,Berhe*etal.(*2007)inTigrayregion,DeselegnandGangwar(2011)inAselladairyfarm,Asgedom*etal.*(2016)inandaroundAlagedistrictswhoreportedhigherprevalenceinfemalethanmale.Thelowerprevalenceofmalereactorsinthisstudycouldbeduetosmallernumberofmalestestedascompared tofemaleandithasalsobeenreportedthattheorganismprefergraviduterusforgrowthandmultiplicationrelativetotesticleandepididymis(Megersa*etal*.,2011).

Inthisstudy,allinfectedanimalswereadultandtherewasstatisticallysignificantdifference(P=0.014)inseroprevalenceof*Brucella*amongdifferentagegroups.Thefindingwasinagreementwith Asgedom*etal.*(2016);Megersa*etal.*(2011)andTsegay*etal.*(2015).Thisfindingwasalsoinagreementwiththereport of Lidia(2008)incentralhighlandofEthiopiaandNuraddis*etal.*(2010)insele

ctedsiteofJimmazone,whoreportedonlyolderagecategoryreactors.Accordingtosomeauthors(Bekele*etal*.,2000;Taye,2005;Roba,2017;Yohannes,2017)susceptibilitytobrucellosisisreportedtoincreaseastheanimalsapproachtothebreedingage.Thus,sexuallymaturecattlearemoresusceptibletoinfectionwith*Brucella*organismthansexuallyimmatureanimalofeithersex(Taye,2005).Inthisstudytherewasnoseropositivereactorinnullparousandinanimalslessthan3yearsofage.Thisfindingwasinagreementwiththeprevalencereportof0.0%innullparousanimalsby(Ibrahim*etal*.,2010),(Berhe*etal*.,2007),(Kebede*etal*.,2008).Thisshowsthatbrucellosisishighlyrelatedwithageandsexualmaturityofanimals.

Breeddifferencesinsusceptibilityhavenotbeenclearlydocumentedincattle,althoughgeneticallydetermineddifferencesinsusceptibilityofindividualanimalshavebeendemonstrated(Yohannes,2017).Inthisstudy,theseroprevalencewasfoundtobehigherinlocal(1.38%)thancrossbreed(0.79%).Thiscouldbedueto,limitednumberofcrossbreedanimalsinthisstudy.Nevertheless,thisdifferencewasnotstaticallysignificantwhichisinagreementwiththereportof(Lidia,2008)and(Yohannes*etal.*,2012)incentralhighlandandEastWollegazoneofEthiopiarespectively.Onthecontrary,Jergefa*etal*.(2009)intheirstudyfoundthatbreedofcattlehassignificanteffectontheserologicalprevalenceofbrucellosisandishigherincrossbreedthaninindigenousones.

Herdsizeremainedsignificantlyassociatedwithseropositivitytobrucellosisinthisstudy.Thisfindingwasinagreementwiththereports(Asmare*etal*.,2010;Hailesillasie*etal*.,2010;Ibrahim*etal*.,2010;Adugna*etal.*,2013;Yohannes,2017).Anincreaseinherdsizeisusuallyaccompaniedbyincreaseinstockingdensity,aswellasanincreaseinriskofexposuretoinfection.Stockingdensityisanimportantdeterminantofthepotentialfortransmissionbetweensusceptibleandinfectedanimals(Omer*etal.*,2000).Itisalsoundeniablefactthatthespreadofthediseasefromoneherdtoanotherherdandfromoneareatoanotherisalmostfrequentlyduetothemovementofaninfectedanimalfromaninfectedherdtoanon-infectedsusceptibleherd(Radostits*etal.*,2000).Thus,brucellosisshouldneverbeviewedasthediseaseofindividualanimals,butshouldbeconsideredinthecontextofherdandalsotheanimalpopulationintheregion.

Thepregnancystatuswassignificantlydeterminingseropositivityinthepresentstudy(p=0.009).Seropositivitytobrucellosiswashigherinpregnantanimals(9%)comparedtonon-pregnantanimals(heifer,lactatinganddrycows)(0.69%).Furthermore,femalecattlearemoresusceptibleto*Brucella*organismingraviduterusofpregnantanimalsduetothepresenceoferythritolinfemalereproductivetractwhichstimulatesthegrowthoftheorganism(Radostits*etal*.,2000).Thi

sdisagreeswiththereportof(Omer*etal*.,2000;Adugna*etal*.,2013;Yohannes,2017)intheirstudyfoundthatpregnancystatusofcattlehasnosignificanteffectontheseroprevalenceofbrucellosis.

Thoughinthepresentstudytheseroprevalenceofbovinebrucellosiswasnotstatisticallysignificantbetweenthemanagementsystems,theresultshowedthatrelativelyhigherseroprevalenceintheextensivemanagementsystem.Insuchcircumstances,cattleofunknowndiseasestatusmightmixandoftengrazedtogetherandresultedinspreadingandtransmissionofdiseaseamongherds.About85%oftheherdsinthestudyareasharedthecommunalgrazingsystem.Ithasalsobeenindicatedthatfreegrazingwhichallowsunrestrictedcontactbetweenanimalsmayhavecontributiontothespreadofbrucellosisinextensivemanagementsystem(Abubakar*etal*.,2012).Thelowerprevalencerecordedinthesemi-intensivemanagementsysteminthestudyareacouldbeduetothebetterhygienicpracticesinthesemi-intensivemanagementsystemwhichwasexpressedbytherelativelybetterproportionoffarmershavingseparateparturitionpens,separatingcowsduringparturition,properlydisposingafterbirthandhavingbetterknowledgeoncauseofabortionandaboutdisease.Brucellosishasbeenlabeledtobeadiseaseofpoorhygienicconditionthatcouldarisesfromexposuretoabortedfetus,placentas,vaginaldischargesornewborncalvesfrominfectedcows(Radostits*etal*.,2000).

Twentypercent(20%)ofanimalswithhistoryofpreviousabortionhad*Brucella*antibodyintheirserumaccordingtorecentstudy.Statisticalanalysisalsorevealedassociationbetween*Brucella*seropositivityandhistoryofpreviousabortion(P=0.001).Associationbetweenbrucellosisseroprevalenceandoccurrenceofabortionalsoreported(Berhe*etal*.,2007;Ibrahim*etal*.,2010;Adugna*etal*.,2013;Tsegaye*etal*.,2016;Yohannes,H.,2017).

Thequestionnairesurveyhasprovidedinformationregardingthesocio-demographiccharacteristicsoftherespondents,farmmanagementandhusbandrypractice,knowledge-attitudeandpracticesofcattleownersaboutbrucellosisinselecteddistrictsofEastWollegazone.Knowledgeofdiseasesisacrucialstepinthedevelopmentofpreventionandcontrolmeasures(Gesse*etal*.,2014).Despitehugeeffortsofthegovernmentinstitutionstoimproveanimalproductioninthareas,mostfarmerswerenotfamiliarizedwithnewtechnologies.Theeducationalstatusattainedbymajorityoftherespondentswaslowwhichfallsbetweenilliterateandlowergrades.Thislowlevelofeducationalstatusmayleadtoreducedproductiongainedfromanimalsbecauseoflowuseofinnovationssuchasartificialinseminationforbreeding,useofmoderndairyfarmingandvaccinat

ionofanimals.Inaddiontothis,properdisposalofabortedmaterials,housinganimalsincorral,us eofaseparateparturitionpenandassistingparturitionbyusingprotectivegloveswerenotunderco nsideration.Thesecouldhaveeffectonthetransmissionofthediseasewithinandbetweentheherds andhuman.Thisisinagreementwithpreviousstudiesinextensivelivestockproductionsystem(R agassa*etal*.2009;Megersa*etal*.2011;Adugna*etal*.,2013).Theoccurrenceofbrucellosisinhuma nsisassociatedwithcontactwithdomesticanimals,exposuretoabortedanimalsandassistingani malparturition(Kozukeev*etal*.2006).Inthisstudy,themajorityoftherespondentshavethehabito fdrinkingrawmilk,rawmeatandassistingparturition.Thisimpliesthelackofawarenessaboutthe effectsofdiseaseandthisinturn,contributestothespreadandtransmissionoftheinfectiontohuma ninthearea.

6.CONCLUSIONANDRECOMMENDATIONS

Resultsofthepresentstudyrevealedthatbovinebrucellosiswasfoundtobe1.23%and6.9%atani mallevelandherdlevelrespectively.ThisindicatesexistenceofbovinebrucellosisintheEastWoll egazoneofOromiaregion,WesternEthiopia.Thefindingofpositiveserologicalreactorsindicate sthepresenceoffociofinfectionthatcouldserveassourcesofinfectionforthespreadofthediseasei ntounaffectedanimalsandherds.Thestudyrevealedthatherdsize,ageofananimal,pregnancystat usofanimalsandabortioncasewerefoundtobesignificantlyassociatedwith*Brucella*seropositivi ty.Thestudiesalsoclearlyshowedthatfarmownershadlessknowledgeofthediseaseandareatrisk ofacquiringtheinfectionthatwasrealizedbyconsumingrawmilk,assistingparturitionandhandli ngofabortedmaterialswithoutusingprotectivegloves.

Basedontheaboveconclusions,thefollowingrecommendationswereforwarded:

- Isolationofcalvinganimalsinseparatecalvingpensshouldbeencouraged.
- Incineratingabortedfetusesandfetalmembraneshouldbepracticed.
- Isolationofdifferentherdsanddifferentspeciesofanimalsshouldbecarriedouttoreduces tockingdensity.
- Properhygienicandgoodmanagementpracticeshouldbeexercised.
- Awarenesscreationforthestakeholdersabouttherouteoftransmission,severityofthedis ease,anditseffectbothonanimalandhumanshouldbeprovided.
- Furthernation-wideandintegratedinvestigationsinallproductionsystemsofdifferentgeographicalarea shouldbeconductedtohaveclearimageonthemagnitudeanddistributionofthedisease.

7.REFERANCES

Abernethy,D.A.,Menzies,F.D.,McCullough,S.J.,McDowell,S.W.J.,Burns,K.E.,Watt,R.,Gordon,A.W.,Greiner,M.andPfeiffer,D.U.,2012.Fieldtrialofsixserologicaltestsforbovinebrucellosis.The*Vet.Journal*,**191**(3),pp.364-370.

Abubakar,M.,Mansoor,M.andArshed,M.J.,2012.BovineBrucellosis:OldandNewConceptswithPakistanPerspective.*Pak.Vet.Journal*,**32**(2).

Acha,P.N.andSzyfres,B.,2001.Zoonosesandcommunicablediseasescommontomanandanimals.*PanAmer.HealthOrg.Reg.OfficeoftheWHO,Washington,USA*,**580**(1):384.

Addis,M.,2015.Publichealthandeconomicimportanceofbrucellosis:Areview.PublicHealth,**5**(7),pp.68-84.

Adone,R.andPasquali,P.,2013.Epidemiosurveillanceofbrucellosis.*RevSciTech*,**32**(1),pp.199-205.

Adugna,K.E.,Agga,G.E.andZewde,G.,2013.Seroepidemiologicalsurveyofbovinebrucellosisincattleunderatraditionalproductionsysteminwestern Ethiopia.*RevSciTech*,**32**(3),pp.765-73.

Agasthya,A.S.,Isloor,S.andKrishnamsetty,P.,2012.Seroprevalencestudyofhumanbrucellosisbyconventionaltestsandindigenousindirectenzyme-linkedimmunosorbentassay.*TheSci.Wrld,J.*,2012.

AlDahouk,S.,Sprague,L.D.andNeubauer,H.,2013.Newdevelopmentsinthediagnosticproceduresforzoonoticbrucellosisinhumans.*RevSciTech*,**32**(1),pp.177-88.

Alem,W.andSolomon,G.,2002.Aretrospectivesero-epidemiologystudyofBovineBrucellosisindifferentProductionSystemsinEthiopia.In*Proceedingof16thannualconference*,pp.53-57.

Álvarez,J.,Sáez,J.L.,García,N.,Serrat,C.,Pérez-Sancho,M.,González,S.,Ortega,M.J.,Gou,J.,Carbajo,L.,Garrido,F.andGoyache,J.,2011.ManagementofanoutbreakofbrucellosisduetoB.melitensisindairycattleinSpain.*ResearchinVet.science*,**90**(2),pp.208-211.

Araj,G.F.,2010.Updateonlaboratorydiagnosisofhumanbrucellosis.*Int.J.Antimicr.Agents,***36**, pp.12-17.

Asgedom,H.,Abdi,D.,andKiros,A.,2016.AReviewonBovineBrucellosis:Epidemiology,Diagnosisand ControlOptions.*ARCJ.ofAnimalandVet.Sciences(AJAVS)*Volume**2**,PP8-21.

AsmareK,SibhatB,MollaW,AyeletG,ShiferawJ,MartinAD,SkjerveEandGodfroidJ.,2013.Thestatusofbovinebrucellosisin Ethiopiawithspecialemphasisonexoticandcrossbredcattleindairyandbreedingfarms.ActaTropica,**126**,pp.186-192.

Asmare,K.,Asfaw,Y.,Gelaye,E.andAyelet,G.,2010.Brucellosisinextensivemanagementsystemof ZebucattleinSidamaZone,SouthernEthiopia.*Afr.J.Agric.Res.***5**(3),pp.257-263.

Baddour,M.M.(2012).Diagnosisofbrucellosisinhumans.*J.Vet.Adv.*,**2**(4):149-156

Bashitu,L.,Afera,B.,Tuli,G.andAklilu,F.,2015.SeroPrevalencestudyofbovinebrucellosisandits associatedriskfactorsinDebrebirhanandAmbotowns.*JAdvDairyRes*,**3**(131),p.2.

Bekele,A.,Molla,B.,Asfaw,Y.andYigezu,L.,2000.BovinebrucellosisinranchesandfarmsinSouth-easternEthiopia.*BulletinofAnim.hlthandProd.inAfr.***48**(1),pp.13-17.

Berhe,G.,Belihu,K.andAsfaw,Y.,2007.Sero-epidemiologicalinvestigationofbovinebrucellosisintheextensivecattleproductionsystemofTigrayregionofEthiopia.*IntJApplResVetMed*.**5**(2),p.65.

Beruktayet,W.andMersha,C.,2016.ReviewofCattleBrucellosisinEthiopia.*Acad.J.Anim.Dis*, **5**(2),pp.28-39.

Birhan,G.,Alebie,A.,Admassu,B.,Shite,A.,Mohamed,S.andDagnaw,B.,2015.AReviewonEmergingandreEmergingViralZoonoticDiseases.

Bosilkovski,M.,Krteva,L.,Dimzova,M.andKondova,I.,2007.Brucellosisin418patientsfromtheBalkanPeninsula:exposure-relateddifferencesinclinicalmanifestations,laboratorytestresults,andtherapyoutcome.*Int.J.ofInfectiousDiseases,***11**(4),pp.342-347.

Boyazoglu,J.,1998.Livestockfarmingasafactorofenvironmental,socialandeconomicstability withspecialreferencetoresearch.*Liv.Prod.Scien.***57**(1),pp.1-14.

Bricker,B.J.,2002.Diagnosticstrategiesusedfortheidentificationof*Brucella*.*Vet.Microbiol.*,**90**(1-4),pp.433-434.

BTWOARD.,2017.BakoTibeWoredaOfficeofAgricultureandRuralDevelopment,AnimalpopulationInformationDesks(BTWOARD).

Buzgan,T.,Karahocagil,M.K.,Irmak,H.,Baran,A.I.,Karsen,H.,Evirgen,O.andAkdeniz,H.,2010.Clinicalmanifestationsandcomplicationsin1028casesofbrucellosis:aretrospectiveevaluationandreviewoftheliterature.*Int.J.Infect.Dis.***14**(6),pp.469-478.

Bwala,D.G.,McCrindle,C.,Fasina,F.O.andIjagbone,I.,2015.Abattoircharacteristicsandserop

revalenceofbovinebrucellosisincattleslaughteredatBodijaMunicipalAbattoir,Ibadan,Nigeria.*J.Vet.Med.Anim.Hlth.*,**7**(5),pp.164-168.

Cadmus,S.I.,Alabi,P.I.,Adesokan,H.K.,Dale,E.J.andStack,J.A.,2013.Serologicalinvestigationofbovinebrucellosisinthreecattleproductionsystemsin YewaDivision,south-westernNigeria.*J.oftheSouthAfricanVet.Association*,**84**(1),pp.12-18.

Cadmus,S.I.B.,Ijagbone,I.F.,Oputa,H.E.,Adesokan,H.K.andStack,J.A.,2006.Serologicalsurveyofbrucellosisinlivestockanimalsandworkersin Ibadan,Nigeria.*Afr.Biom.Research*,**9**(3).

Chain,P.S.,Comerci,D.J.,Tolmasky,M.E.,Larimer,F.W.,Malfatti,S.A.,Vergez,L.M.,Aguero,F.,Land,M.L.,Ugalde,R.A.andGarcia,E.,2005.Whole-genomeanalysesofspeciationeventsinpathogenicBrucellae.*Inf.andImm.*,**73**(12),pp.8353-8361.

Chauhan,H.C.,Patel,K.B.,Patel,S.I.,Patel,B.K.,Chandel,B.S.,Bhagat,A.G.,Patel,M.V.,Patel,S.S.,Patel,A.C.,Shrimali,M.D.andShome,R.,2017.SerologicalSurveyofBrucellosisinCamelofGujarat.*Inter.J.ofCurrentMicrobiologyandAppliedSciences*,**6**(4),pp.1815-1821.

Cheville,N.F.,McCullough,D.R.,Paulson,L.R.andNationalResearchCouncil,1998.*Brucellosisinthegreater Yellowstonearea*.NationalAcademiesPress.

Chugh,T.D.,2008.Emergingandre-emergingbacterialdiseasesinIndia.*J.Biosci.*,**33**(4),pp.549-555.

Chukwu,C.C.,1985.BrucellosisinAfrica.PartI:Theprevalence.*BulletinofAnim.HlthandProd.inAfr*.**33**:193-198.

Colmenero,J.D.,Morata,P.,Ruiz-Mesa,J.D.,Bautista,D.,Bermúdez,P.,Bravo,M.J.andQueipo-Ortuño,M.I.,2010.Multiplexreal-timepolymerasechainreaction:apracticalapproachforrapiddiagnosisoftuberculousandbrucellarvertebralosteomyelitis.*Spine*,**35**(24),pp.1392-1396.

CSA(CentralStatisticalAgencyofEthiopia)AgriculturalSampleSurvey2012/13.Areportonlivestockandlivestockcharacteristics,570statisticlbulletin,AddisAbaba,2:37.

Degefa,T.,Duressa,A.andDuguma,R.,2011.Brucellosisandsomereproductiveproblemsofindigenous Arsicattleinselected Arsizones of Oromiaregionalstate,Ethiopia.*GlobalVeterinaria*,**7**(1),pp.45-53.

Degefu,H.,Mohamud,M.,Hailemelekot,M.andYohannes,M.,2011.Seroprevalenceofbovinebrucellosisinagropastoralareasof Jijjigazone of SomaliNationalRegionalState,EasternEthiopia.*Eth.Vet.J.*,**15**(1).

Deselegn,T.B.&Gangwar,S.K.(2011):SeroprevalencestudyofbovinebrucellosisinAsselagovernmentdairyfarmofOromiaRegionalState,Ethiopia.Shortcommunication,*Int.J.Sci.Natr.*,**2**(3):692-697.

DiFebo,T.,Luciani,M.,Portanti,O.,Bonfini,B.,Lelli,R.andTittarelli,M.,2012.Developmentan devaluationofdiagnostictestsfortheserologicaldiagnosisofbrucellosisinswine.*VetIta l*,**48**(2),pp.133-156.

Dinka,H.andChala,R.,2009.Seroprevalencestudyofbovinebrucellosisinpastoralandagro-pastoralareasofEastShowaZone,OromiaRegionalState,Ethiopia.*Amer.EurasianAgr icEnvironSci*,**6**(5),pp.508-12.

Ferede,Y.,Mengesha,D.andMekonen,G.,2011.Studyontheseroprevalenceofsmallruminantb rucellosisinandaroundBahirDar,NorthWestEthiopia.*Ethiop.Vet.J.*,**15**(2).

Folitse,R.D.,Boi-Kikimoto,B.B.,Emikpe,B.O.andAtawalna,J.,2014.TheprevalenceofBovinetubercul osisandbrucellosisincattlefromselectedherdsinDormaaandKintampoDistricts,Bron gAhaforegion,Ghana.*ArchivesofClin.Microb.*,**5**(2).

Foster,G.,Osterman,S.B.,Godfroid,J.andJacques,I.,2007.A.*Brucellaceti*sp.nov.and*Brucella pinnipedialis*sp.nov.for*Brucella*strainswithcetaceansandsealsastheirpreferredhosts. *Int.J.Syst.Evol.Microbiol.***57**:2688–2693.

Gall,D.andNielsen,K.,2004.Serologicaldiagnosisofbovinebrucellosis:areviewoftestperform anceandcostcomparison.*Rev.Sci.Tech.*,**23***:989-1002.*

GBWOARD.,2017.GudayaBilaWoredaOfficeofAgricultureandRuralDevelopment,Animal populationInformationDesks(GBWOARD).

Georgi,E.,Walter,M.C.,Pfalzgraf,M.T.,Northoff,B.H.,Holdt,L.M.,Scholz,H.C.,Zoeller,L.,Z ange,S.andAntwerpen,M.H.,2017.WholegenomesequencingofBrucellamelitensisis olatedfrom57patientsinGermanyrevealshighdiversityinstrainsfromMiddleEast.*PLo SOne*,**12**(4),p.e0175425.

Geresu,M.A.,AmeniG.,KassaT.,TuliG.,ArenasA.andKassa,G.M.,2016.Seropositivityandris kfactorsfor*Brucella*indairycowsinAsellaandBishoftutowns,OromiaRegionalState,E thiopia.*Afr.J.ofMicr.Research*,**10**(7):203-213.

Gessese,A.T.,Mulate,B.,Nazir,S.andAsmare,A.,2014.Seroprevalenceofbrucellosisincamels (Camelusdromedaries)inSouthEastEthiopia.*JVetSciMedDiagn***3**(1):2.

Ghanem,Y.M.,El-Khodery,S.A.,Saad,A.A.,Abdelkader,A.H.,Heybe,A.andMusse,Y.A.,2009.Seropre valenceofcamelbrucellosis(Camelusdromedarius)inSomaliland.*Trop.Anim.Hlthan dProd.***41**:1779–1786.

Godfroid,H.C.,S.T.Barbier,C.Nicolas,P.,Wattiau,D.,Fretin,A.M.,Whatmore,A.,Cloeckaert, J.M.,Blasco,I.,Moriyon,C.,Saegerman,J.,Muma,B.,AlDahouk,S.andLetesson,J.J.,2 011.Brucellosisattheanimal/ecosystem/humaninterfaceatthebeginningofthe21stcen tury.*Prev.Vet.Med.***102:**(2),118–131.

Godfroid,J.,AlDahouk,S.,Pappas,G.,Roth,F.,Matope,G.,Muma,J.,Marcotty,T.,Pfeiffer,D.an dSkjerve,E.,2013.A“OneHealth”surveillanceandcontrolofbrucellosisindevelopingc

ountries:movingawayfromimprovisation.*Comparativeimmunology,micr.andinfect. diseases*,***36***(3),pp.241-248.

Godfroid,J.,Bosman,P.P.,Herr,S.,Bishop.G.C.,2004.Bovinebrucellosis.In:CoetzerJ.A.W,T ustinR.C(eds).*InfectiousDiseaseofLivestock*,2nd Edition,Vol.3OxfordUniversityPres s:1510-1527.

Godfroid,J.,Cloeckaert,A.,Liautard,J.P.,Kohler,S.,Fretin,D.,Walravens,K.,Garin-Bastuji,BandLetesson,J.J.,2005.FromthediscoveryoftheMaltafever'sagenttothedisc overyofamarinemammalreservoir,brucellosishascontinuouslybeenare-emergingzoonosis.*Vet.Res*.**36**:313–326.

GSWOARD.,2017.GobuSeyoWoredaOfficeofAgricultureandRuralDevelopment,Animalp opulationInformationDesks(GSWOARD).

Haghi,F.,Zeighami,H.,Naderi,G.,Samei,A.,Roudashti,S.,Bahari,S.andShirmast,P.,2015.Det ectionofmajorfood-bornepathogensinrawmilksamplesfromdairybovineandovineherdsinIran.*SmallRum .Res*.***131***,pp.136-140.

Haileselassie,M.,Shewit,K.andMoses,K.,2010.Serologicalsurveyofbovinebrucellosisinbark aandaradobreeds(Bosindicus)ofWesternTigray,Ethiopia.*Prev.Vet.Medicine*,**94**(1-2):28-35.

Hilemlekot,M.,2005.SeroprevalencestudyofbrucellosisincattleandhumaninBahrDarmilkshe d,FVM,AAU,Debre-zeit,Ethiopia.

Holveck,J.C.,Ehrenberg,P.J.,Ault,K.S.,Rojas,R.,Vasquez,J.,Cerqueira,M.T.,Shepherd,I.J., Genovese,M.A.andPeriago,R.M.,2007.Prevention,controlandeliminationofneglect eddiseasesintheAmericas:Pathwaystointegrated,inter-programmatic,inter-sectoralactionforhealthanddevelopment.*BMCPublicHealth*,**7**(6):1471-2458.

Ibrahim,N.,Belihu,K.,Lobago,F.andBekana,M.,2010.Seroprevalenceofbovinebrucellosisan ditsriskfactorsinJimmazoneofOromiaregion,South-westernEthiopia.*Trop.Anim.HealthProd*.**42**:35-40.

James,L.W.,2013.StudiesonhumanbrucellosisintheMikumiselousecosystem,Morogoro,Tan zania(Doctoraldissertation,SokoineUniversityofAgriculture).

Jergefa,T.,Kelay,B.,Bekana,M.,Teshale,S.,Gustafson,H.andKindahl,H.,2009.Epidemiologi calstudyofbovinebrucellosisinthreeagro-ecologicalareasofcentralOromiya,Ethiopia.*J.ofAgr.andEnv.Science*,**6**(5):508-512.

Kambarage,D.M.,Karimuribo,E.D.,Kusiluka,L.J.M.,Mdegela,R.H.andKazwala,R.R.,2003. CommunitypublichealtheducationinTanzania:Challenges,opportunitiesandthewayf orward.*ExpertConsultationonCommunityBasedVeterinaryPublicHealth(VPH)Syst ems*,p.9.

Kang'ethe,E.K.,Ekuttan,C.E.,Kimani,V.N.,Kiragu,M.W.,2007.Investigationsintotheprevale nceofbovinebrucellosisandtheriskfactorsthatpredisposehumanstoinfectionamongur bandairyandnon-

dairyfarminghouseholdsinDagorettiDivision,Nairobi,Kenya.*EastAfr.Med.J.*,**84**:96-100.

KassahunA.,2007.Epidemiologyofbrucellosisincattleanditssero-epidemiologyinanimalhealthprofessionalsinSidamazone,South,**MScTesis**,FVM,AAU,Debrezeit,Ethiopia.

Kassahun,J.,Yimer,E.,Geyid,A.,Abebe,P.,Newayeselassie,B.,Zewdie,B.,Beyene,M.andBekele,A.,2006.Sero-prevalenceofbrucellosisinoccupationallyexposedpeopleinAddisAbaba,Ethiopia.*Eth.Med.Journal*,**44**(3),pp.245-252.

Kebede,T.,Ejeta,G.andAmeni,G.,2008.Seroprevalenceofbovinebrucellosisinsmallholderfarmsincentral Ethiopia(Wuchale-Jidadistrict).*Rev.Méd.Vet.*,**159**:3-9.

Khan,M.andZahoor,M.,2018.Anoverviewofbrucellosisincattleandhumans,anditsserologicalandmoleculardiagnosisincontrolstrategies.*Trop.Med.andinfectiousdisease*,**3**(2),p.65.

Kozukeev,T.B.,Ajeilat,S.,Maes,E.,Favorov,M..2006.CentersforDiseaseControl,Prevention (CDC).Riskfactorsforbrucellosis,**1**:31–34.

Lado,D.,Maina,N.,Lado,M.,Abade,A.,Amwayi,S.,Omolo,J.andOundo,J.,2012.Brucellosisin TerekekaCounty,CentralEquatoriaState,SouthernSudan.*EastAfri.MedicalJ.*,**89(1):**28-33.

Lapaque,N.,Moriyon,I.,Moreno,E.andGorvel,J.P.,2005.*Brucella*lipopolysaccharideactsasa virulencefactor.*CurrOpinMicrobiol*.**8**:60–6.

Lidia,B.,2008.SeroprevalencestudyofbovinebrucellosisinCentralHighLandofEthiopia,**DVMThesis**,JimmaUniversity,Jimma,Ethiopia.

Lim,J.J.,Kim,D.H.,Lee,J.J.,Kim,D.G.andMin,W.,2004.Evaluationofrecombinant28kDaoutermembraneproteinof*Brucellaabortus*fortheclinicaldiagnosisofbovinebrucellosisin Korea.*J.Vet.Med.Sci.*,**74:**687-691.

Maadi,H.,Moharamnejad,H.andHaghi,A.,2011.PrevalenceofbrucellosisincattleinUrmia,Iran.*Pak.Vet.J*.**31**:818–2.

Makita,K.,Fevre,E.M.,Waiswa,C.,Kaboyo,W.,Bronsvoort,D.C.,Eisler,M.C.andWelburn,S.C.,2008.Humanbrucellosisinurbanandperi-urbanareasofKampala,Uganda.AnimalBiodiversityandemergingdisease.*Acd.Sci.Health*,**138:**190-210.

Makita,K.,Fèvre,E.,Waiswa,M.E.,Eisler,C.,Thrusfield,M.C.andWelburn,S.C.,2011.Herdprevalenceofbovinebrucellosisandanalysisofriskfactorsincattleinurbanandperi-urbanareasoftheKampalaeconomiczone,Uganda.*Vet.Res.*,**7**:60.

Mangen,M.J.,Otte,J.,Pfeiffer,D.andChilonda,P.,2002.Bovinebrucellosisinsub-SaharanAfrica:estimationofsero-prevalenceandimpactonmeatandmilkofftakepotential.*FoodandAgr.Org.oftheUnitednations,Rome*.

Mantur,B.G.,Amarnath,S.K.andShinde,R.S.,2007.Reviewofclinicalandlaboratoryfeatureso fhumanbrucellosis.*IndianJ.ofmed.microbiology*,**25**(3),p.188.

Marín,C.M.,DeBagüés,M.J.,Barberán,M.andBlasco,J.M.,1996.Comparisonoftwoselective mediafortheisolationof*Brucellamelitensis*fromnaturallyinfectedsheepandgoats.Vet erinaryrecord,**138**(17):409-411.

Matope,D.G.,Bhehe,E.,Muma,J.B.,Land,A.andSkjerve,E.,2010.Riskfactorsfor*Brucella*spp. infection.*Epidemiol.Infect.**39**:*157–164.

Maurin,M.,2005.Brucellosisatthedawnofthe21stCentury.*Med.Et.Mal.Inf.**35***:6–16.

McDermott,J.JandArimi,S.M.,2002.Brucellosisinsub-SaharanAfrica:epidemiology,controlandeconomicimpact.*Vet.Microb.***90**(1–4):111–134.

McDermott,J.J.,Deng,K.A.,Jayatileka,T.N.andElJack,M.A.,2013.Across-sectionalcattlediseasestudyinKongorRuralCouncil,southernSudan:prevalenceestim atesandage,sexandbreedassociationsforbrucellosisandcontagiousbovinepleuropneu monia.*PreventiveVeterinaryMedicine*,**5:**111-123.

McGiven,J.A.,Tucker,J.D.,Perrett,L.L.andStack,J.A.,2003.ValidationofFPAandc-ELISAfortheDetectionofAntibodiesto*Brucellaabortus*inCattleSeraandComparison toSAT,CFT,andi-ELISA.*J.Immunol.Methds*,**278**(1-2):171-178.

Megersa,B.,BiffaD.,Niguse,F.,Rufael,T.,Asmare,K.andSkjerve,E.,2011.Cattlebrucellosisin traditionallivestockhusbandrypracticeinSouthernandEasternEthiopia,anditszoonoti cimplication.*ActaVetScand*53:24

Megid,J.,Mathias,L.A.andRobles,C.A.,2010.Clinicalmanifestationsofbrucellosisindomesti canimalsandhumans.*Open.Vet.Sci.J.***4**:119–126.

MerckVeterinaryManual,2012.BrucellosisinCattle.

Meyer,C.E.,1980.ReportonVeterinaryactivities.InstituteofAgriculturalResearch,Ethiopia.F AOReportNo.AG:DP/ETH/78/004.FAO(FoodandAgricultureOrganizationoftheUn itedNations),Rome,Haly,pp:24.

Mitika,S.,Anetakis,C.,Souliou,E.,Diza,E.andKansouzidou,A.,2007.EvaluationofdifferentP CRassaysforearlydetectionofacuteandrelapsingbrucellosisinhumansincomparison withconventionalmethods.*J.Clin.Microbiol.*,**45:**1211-1218.

MoA(MinistryofAgriculture)2012/2004E.C.Performanceassessmentreportonthegrowthand transformationagendainthespheresofagriculture.

Molyneux,D.,Hallaj,Z.,Keusch,G.T.,McManus,D.P.,Ngowi,H.,Cleaveland,S.,Ramos-Jimenez,P.,Gotuzzo,E.,Kar,K.,Sanchez,A.andGarba,A.,2011.Zoonosesandmargina lisedinfectiousdiseasesofpoverty:*Parasites&vectors*,**4**(1),p.106.

Moreno,E.,Cloeckaert,A.andMoriyon,I.,2002.*Brucella*evolutionandtaxonomy.*VetMicrobio l.*;**90**:209–227.

Muendo,E..,Mbatha,P.M.,Macharia,J.,Abdoel,T.H.,Janszen,P.V,Pastoor,R.andSmits,H.L., 2012.InfectionofcattleinKenyawith*Brucellaabortus*biovar3and*Brucellamelitensis*b iovar1genotypes.*Trop.Anim.HealthProd*.**44**:17-20.

Muflihanah,H.,Hatta,M.,Rood,E.,Scheelbeek,P.,Abdoel,T.H.andSmits,H.L.,2013.Brucello sisseroprevalenceinBalicattlewithreproductivefailureinSouthSulawesiand*Brucella abortus*biovar1genotypesintheEasternIndonesianarchipelago.*BMCVetRes*;**9**:233.

Muma,J.B.,Samui,K.L.,Siamudaala,V.M.,Oloya,J.,Matope,G.,Omer,M.K.,Munyeme,M., Mubita,C.andSkjerve,E.,2014.Prevalenceofantibodiesto*Brucellaspp*.andindividual riskfactorsofinfectionintraditionalcattle,goatsandsheepreparedinlivestock-wildlifeinterfaceareasofZambia.*Trop.Anim.HlthandProd*.**38**:195–206.

Muma,J.B.,Syakalima,M.,Munyeme,M.,Zulu,V.C.,Simuunza,M.andKurata,M.,2013.Bovin etuberculosisandbrucellosisintraditionallymanagedlivestockinSelectedDistrictsofS outhernProvinceofZambia.VeterinaryMedicineInternational,HindawiPublishingCo rporation.

Munir,R.,Afzal,M.,Hussain,S.M.,Naqvi,S.andKhanum,A.,2010.Outermembraneproteinsof *B.abortus*vaccinalandfieldstrainsandtheirimmuneresponseinbuffaloes.*Pak.Vet.J*.**30** :110–114

Munoz,P.,Marin,C.,Monreal,D.,Gonzales,D.,Garin-Bastuji,B.,Diaz,R.,Mainar-Jaime,R.,Moriyon,I.andBlasco,J.,2005.Efficacyofseveralserologicaltestsandantige nsforthediagnosisofbovinebrucellosisinthepresenceoffalsepositiveserologicalresult sdueto*YersiniaenterocoliticaO:9.Clin.Diagn.Lab.Immunol.*,**12:**141-151.

Murray,R.G.andHolt,J.G.,2005.ThehistoryofBergey'sManual.*InBergey'sManual®ofSyste maticBacteriology*(pp.1-14).Springer,Boston,MA.

Mussie,H.,2007.Sero-prevalencestudyofbrucellosisincattleandhumaninBahirDarmilkshed.**MscThesis**,F VM,AAU,Debrezeit,Ethiopia.

Neta,A.V.C.,Mol,J.P.,Xavier,M.N.,Paixão,T.A.,Lage,A.P.andSantos,R.L.,2010.Pathogene sisofbovinebrucellosis.*TheVet.J.*,**184**(2):146-155.

Nicoletti,P.andGilsdorf,M.J.,1997.Brucellosis-thediseaseincattle.Pages3-6inE.T.Thorne,M.S.Boyce,P.,NicolettiandT.J.Kreeger,editors-Brucellosis,bison,elkandcattleinthegreaterYellowstonearea,definingtheproblem,ex ploringsolutions.WyominGameandFish,Cheyenne,Wyoming.

Nielsen,K.,2002.DiagnosisofbrucellosisbyserologyVet.*Microbiol.*,**90***:447-459.*

Nielson,O.,Steward,R.,Nielson,K.,Measurs,K.andDuigan,P.,2001.Serologicsurveyof*Bruce lla*Species,antibodiesinsomeanimalsofNorthAmerica.*J.WildlifeDis*.,**37**:89-100.

Nuraddis,I.,Kelay,B.,FikreL.andMerga,B.,2010.Seroprevalenceofbovinebrucellosisanditsri skfactorsinJimmazoneofOromiaregion,southwesternEthiopia.*TropicalAnimalHealt handProduction*,**42**:35-40.

Olano,P.J.andWalker,H.D.,2011.DiagnosingEmergingandRe-

emergingInfectiousDiseasesthePivotalRoleofthePathologist.*ArchivesofPathologyandLaboratoryMedicine*,**135**:83–91.

Omer,K.M.,Skjerve,E.,Woldehiwet,Z.,Holstand,G.,2000.Riskfactorsfor*Brucella*speciesinfectionindairycattlefarmsinAsmara,StateofEritrea.*PreventiveVeterinaryMedicine***46**,257-265.

Pace,J.E.andWakeman,D.L.,1983.Determiningtheageofcattlebytheirteeth.UniversityofFloridaCooperativeExtensionService,InstituteofFoodandAgricultureSciences,EDIS.

PadillaPoester,F.,Klaus,Nielson,K.,Luis,E.andWei,Y.2010.DiagnosisofBrucellosis.*TheOpenVet.Sci.Jour.***4**:46-60.

PAHO/WHO.,2001.ZoonosesandCommunicableDiseasesCommontoManandAnimals.3rdedition.BacteriosisandMycosisScientificandTechnicalPublication,No.580.PanAmericanHealthOrganizationPanAmericanSanitaryBureau,RegionalOfficeoftheWorldHealthOrganization.WashingtonD.C,USA.pp.:57-58.

Pandey,G.S.,Hang'ombe,B.M.,Mushabati,F.andKataba,A.,2013.PrevalenceoftuberculosisamongsouthernZambiancattleandisolationofMycobacteriumbovisinrawmilkobtainedfromtuberculinpositivecows.*Vet.World*,**6**(12):986.

Pappas,G.,Akritidis,N.,Bosilkovski,M.andTsianos,E.,2005.Brucellosis.NewEngl.*J.ofMed.***352**:2325–2336.

Pappas,G.,Papadimitriou,P.,Akritidis,N.,Christou,LandTsianos,E.V.,2006.Thenewglobalmapofhumanbrucellosis.*Lancet.Infect.Dis.***6**:91–99.

Park,M.Y.,Lee,C.S.,Choi,Y.S.andLee,H.B.,2005.Asporadicoutbreakofhumanbrucellosisin Korea.*J.Kor.Med.Sci*.**20**:941–946.

Pieracci,E.G.,Hall,A.J.,Gharpure,R.,Haile,A.,Walelign,E.,Deressa,A.,Bahiru,G.,Kibebe,M.,Walke,H.andBelay,E.,2016.Prioritizingzoonoticdiseasesin Ethiopiausingaonehealth approach.*OneHealth*,***2***,pp.131-135.

Poester,F.P.,Nielsen,K.,andYu,W.L.(2010.DiagnosisofBrucellosis.*OpenVet.Sci.J*.**4**:46.

Poester,F.P.,Samartino,L.E.and.SantosR.L.,2013.PathogenesisandPathobiologyofbrucellosisinlivestock:*Rev.Sci.tech.off.Int.Epiz***,32**(1):105-115.

Queipo-Ortuño,M.I.,ColmeneroJ.D.,MaciasM.,BravoM.J.andMorataP.,2008.PreparationofbacterialDNAtemplatebyboilingandeffectofimmunoglobulinGasaninhibitorinrealtimePCRforserumsamplesfrompatientswithbrucellosis.*Clin.VaccineImmunol*.**15:**293-296.

Quinn,P.J.,Carter,M.E.,Donnely,W.J.C.,Lonard,F.C.andMaquire,D.,2002.*BrucellaSpecies*inVeterinaryMicrobiologyandMicrobialDisease,Londen,BlackwellScienceltd,pp.:999-1000.

Racloz,V.,Schelling,E.,Chitnis,N.,Roth,F.andZinsstag,J.,2013.Persistenceofbrucellosisinpastoralsystems.*OIERevueScientifiqueetTechnique*,***32***(1),pp.61-70.

Radostits,E.D.,Gay,C.C.andHinchcliff,K.W.,2006.VeterinaryMedicineTextbookofthediseaseofCattle,Sheep,Pigs,GoatsandHorses,9th ed.,Newyork:W.B.SundersCompanyLtd.,pp:867-882.

Radostits,O.M.,Gay,C.C.,Blood,C.D.andHinchcliff,K.H.,2000.VeterinaryMedicine,TextbookoftheDiseaseofCattle,Sheep,Pigs,GoatsandHorses.NEWYORK:W.B.SoundersCompanyItd,pp:867-882

Radostits,O.M.,Gay,C.C.,Hinchcliff,K.W.andConstable,P.D.,2007.Veterinarymedicine:atextbookofthediseasesofcattle,horses,sheep,pigsandgoats,10thedn.SaundersElsevier,London

Ragassa,G.,Mekonnen,D.,Yamuah,L.,Tilahun,H.,Guta,T.,Gebreyohannes,A.,Aseffa,A.,Abdoel,T.H.andSmits,H.L.,2009.HumanbrucellosisinTraditionalpastoralcommunitiesinEthiopia.*IntJTropMed***4**:59–64

Ramirez,M.,Hamdy,M.E.,andAmin,M.,2006.Serologicresponseandtimetoeradicationinherdswithbrucellosisvaccinatedwithstrain19orstrainRB-51.Archivosdemedicinaveterinaria**34**:143-151.

Refai,M.,2002.IncidenceandcontrolofbrucellosisintheNearEastregion.*Vet.Microb.***90***:*81-110.report.*WHOTech.Rep.Series*.**464**:1–76.

Reshid,M.,1993.ReproductiveWastageinCattleDuetoBovineBrucellosis.NationalLivestockImprovementConference.AddisAbeba(Ethiopia).13-15Nov1991.

Roba,J.,2017.BrucellosisinBorenacattle:-SeroprevalenceandawarenessofthepastoralcommunityinYabello,Ethiopia(doctoraldissertation).

Robinson,A.,2003.GuidelinesforcoordinatedhumanandanimalbrucellosissurveillanceinFAOAnimalproductionandHealthpaper156.*Rome:FoodandAgricultureOrganization*.

Sathyanarayan,S.,Suresh,S.,Krishna,S.andMariraj,J..2011.Acomparativestudyofagglutinationtests,bloodcultureandELISAinthelaboratorydiagnosisofhumanbrucellosis.*Int.J.Biol.Med.Res.,***2***:569-572*.

Scacchia,M.,Diprovvido,A.,Ippoliti,C.,Kefle,U.,SebhatuT.,Angelo,A.andDeMassis,F.,2013.PrevalenceofbrucellosisindairycattlefromthemaindairyfarmingregionsofEritrea.*J.vet.Res.*,**80**(1):448.

Scholz,H.C.,Hofer,E.,Vergnaud,G.,LeFlèchеP.,WhatmoreA.M.,AlDahouk,S.,Pfeffer,M.,Krüger,M.,Cloeckaert,A.andTomaso,H.,2009.Isolationof*Brucellamicroti*frommandibularlymphnodesofRedfoxes(*Vulpesvulpes)*,inlowerAustria.*Vect-bornezoon.Dis*.**9**(2):153–156.

Scholz,H.C.,Hubalek,Z.,Sedláček,I.,Vergnaud,G.,Tomaso,H.,AlDahouk,S.,Melzer,F.,Kämpfer,P.,Neubauer,H.,Cloeckaert,A.andMaquart,M.,2008.Brucellamicrotisp.nov.,isol

atedfromthecommonvoleMicrotusarvalis.*Int.J.ofsystematicandevolutionarymicrobiology*,***58***(2),pp.375-382.

Scholz,H.C.,Nockler,K.,Gollner,C.,Bahn,P.,Vergnaud,G.,Tomaso,H.,AlDahouk,S.,Kampfer,P.,Cloeckaert,A.,Maquart,M.,Zygmunt,M.S.,Whatmore,A.M.,Pfeffer,M.,Huber,B.,Busse,H.J.andDeB.K.,2010.*Brucella*inopinataisolatedfromabreastimplantinfection.*Int.J.ofSyst.andEvol.Microb.***60**:801–808.

Seleem,M.N.,Boyle,S.M.andSriranganathan,N.,2010.Brucellosis:are-emergingzoonosis.Veterinarymicrobiology,**140**(3-4):392-398.

Senein,M.&Abdelkadir,A.,2012.SerologicalsurveyofcattlebrucellosisinEldein,easternDarfur,Sudan.*Acad.J.*,***6***(31):6086-6090.

Shey-Njila,O.,Daouda,N.E.,Zoli,P.A.,Walravens,K.,Godfroid,J.andGeerts,S.,2005.SerologicalsurveyofbovinebrucellosisinCameroon.Revued"ElevageetdeMedicineVétérinairedesPaysTropicaux,**58**(3):139-143

Shirima,G.M.,Fitzpatrick,J.,Kunda,J.S.,Mfinanga,G.S.,Kazwala,R.R.,KambarageD.M.andCleaveland,S.,2010.TheroleoflivestockkeepinginhumanbrucellosistrendsinlivestockkeepingcommunitiesinTanzania.Shortcommunication.*Tanz.J.Hlth.Res.***1**(3).

Shirima,G.M.,Fitzpatrick,J.,Cleaveland,S.,Kambarage,D.M.,Kazwala,R.R.,Kunda,J.andFrench,N.P.,2003.ParticipatorySurveyonZoonosesAffectingLivestockKeepingCommunitiesinTanzania.*J.ofAnim.andVet.Adv.***4**:253–258.

Singh,B.B.,Dhand,N.K.andGill,J.P.S.,2015.EconomiclossesoccurringduetobrucellosisinIndianlivestockpopulations.*Prevent.Vet.medicine*,**119**(3-4),pp.211-215.

Sintaro,T.,1994.TheimpactofbrucellosisonproductivityinanimproveddairyherdofChaffastatefarm,Ethiopia.FachburgVeterinaemedizin,FreiUniversitate,Berlin,**MscThesis**.

Smits,H.L.andCutler,S.J.,2004.ContributionsofbiotechnologytothecontrolandinpreventionofbrucellosisinAfrica.*AfricanJournalofBiotechnology*,**3**(12):631.

Smits,L.S.and.Kadri,M.,2005.BrucellosisinIndia:adeceptiveinfectiousdisease.*IndianJournalofMedicalResearch*,**122**:375-384.

Tadese,Y.,2003.AsurveyofbrucellosisinselectedareaofNorthGonderzone,**DVMThesis**,AddisAbabaUniversity,DebreZeit,Ethiopia.

Taye,K.A.,2005.Crosssectionalstudyofbovinebrucellosisinsmallholderfarmsin Salale.**DVM Thesis**,AddisAbabaUniversity,Debrezeit,Ethiopia.

Tefera,M..,2006.StudyonbovinebrucellosisincattleslaughteredatAddisAbabaandSululta with focusonoccupationalhazard,FVM,AAU,Debre-zeit,Ethiopia.

Tesfaye,G.,Tsegaye,W.,Chanie,M.andAbinet,F.,2011.SeroprevalenceandassociatedriskfactorsofbovinebrucellosisinAddisAbabadairyfarms.*TropicalAnimalHealthandProduction*,**43**:1001-1005.

Thakur,S.D.andThapliyalD.C.,2002.Seroprevalenceofbrucellosisinman.*J.Commun.Dis.*,**34**: 106-109.

Thrusfield,M.,2007.Samplesizedetermination.VeterinaryEpidemiology,*3*,pp.185-189.

Tolosa,T.,2004.Seroprevalencestudyofbovinebrucellosisanditspublichealthsignificanceinselectedsitesof JimmaZone,WesternEthiopia.Ethiopia:**MscThesis,**FVM,AAU.Debrezeit,Ethiopia.

Tsegaye,Y.Kyuleb,M.andLobagob,F.,2016.SeroprevalenceandRiskFactorsofBovinebrucellosisinArsiZone,OromiaRegionalState,Ethiopia.*AmericanSci.Res.J.Engin.,Technol. Sci.*,**24***:16-25.*

Unger,F.,Mnstermann,S.,Goumou,A.,Apia,C.N.,Konte,M.andHempen,M.,2003.Riskassociatedwithbovinebrucellosisinselectedstudyherdsandmarketplacesinfourcountriesof WestAfricaanimalhealthworkingpaper2.International*Trypanotolerance*Centre,Banjul,Gambia.

Wadood,F.,Ahmad,M.,Khan,A.,Gul,T.andRehman,N.,2009.Seroprevalenceofbrucellosisin horsesinandaroundFaisalabad.*Pak.Vet.J.*,**29:**196-198.

WorldBank/TrustinAnimalsandFoodSafety(TAFS)Forum,2011.Worldlivestockdiseaseatlas:aquantitativeanalysisofglobalanimalhealthdata(2006–2009).WorldBank.

WorldHealthOrganization(WHO),2006.EmergingandCommunicableDiseasesurveillanceandcontrol.Thedevelopmentofnewbrucellosis.ReportoftheWHOmeetings,Geneva,December,pp:41-47.

WorldOrganisationforAnimalHealth(OIE)(2012).Bovinebrucellosis.In:OIEManualofdiagnostictestsandvaccinesforterrestrialanimals.Paris:OfficeInternationaldesEpizooties, p.616.

WorldOrganisationforAnimalHealth(OIE),2016.Bovinebrucellosis:OIEManualofdiagnostictestsandvaccinesforterrestrialanimals.Paris:OfficeInternationaldesEpizooties,p.616.

WorldOrganizationforAnimalHealth(OIE),2000.OvineEpididimytis(*B.ovis*)ManualofstandardforDiagnosticTestandVaccines.3rded.OIE,Paris,France,pp:467-474

WorldOrganizationforAnimalHealth(OIE),2004.Manualofdiagnostictestsandvaccinesforterrestrialanimals,5thEd.OIE,Paris,France.

WorldOrganizationforAnimalHealth(OIE),2009.BovineBrucellosis;versionadoptedbytheworldAssemblyofdelegatesoftheOIE,pp:639-640.

WorldorganizationforAnimalHealth(OIE),2010.Bovinebrucellosis,Chapter2.4.3.[VersionadoptedbytheWorldAssemblyofDelegatesoftheOIEinMay2009].InManualofDiagnosticTestsandVaccinesforTerrestrialAnimals.OIE,Paris.

WorldOrganizationforAnimalHealth(OIE),2011.BovineBrucellosis.OIEManualofdiagnosticTestsandVaccinesforTerrestrialAnimals.OIE,Paris.

Xavler,M.N.,PalxaoT.A.,PoesterE.P.,LageA.P.andSantosR.L.,2009.Pathology,immunehistochemistryandbacteriologyoftissuesandmilkofcowsandfetusesexperimentallyinfectedwith*Brucellaabortus*.*J.Comp.Pathol*.,**140:**147-157.

Yifat,D.,Kelay,B.,Bekana,M.,Lobago,F.,Gustafsson,H.andKindahl,H.,2012.Studyonreproductiveperformanceofcrossbreddairycattleundersmallholderconditionsinandaround Zeway,Ethiopia.*Parity*,**277,**pp.0-23.

Yohannes,H.,2017.Seroprevalenceofbovinebrucellosisunderextensiveproductionsystemin wolaitazone,southernethiopia(doctoraldissertation).

Yohannes,M.,Degefu,H.,Tolosa,T.,Belihu,K.,Cutler,R.andCutler,S.,2013.Distributionofbrucellosisindifferentregionsin Ethiopia.*Afric.J.ofMicrob.Research*,**7:**1150-1157.

Yohannes,M.,MershaT,Degefu,H.,TolosaTandWoyesa,M.,2012.SerologicalsurveyinGuto-GidaDistrict,EastWollegazone,Ethiopia.GlobalVeterinaria**8**(2):139–43.

Zamri-Saad,M.andKamarudin,M.I.,2016.Controlofanimalbrucellosis:TheMalaysianexperience.*AsianPac.J.Trop.Med*.**9**:1136-1140.

Zewdie,W.,2018.ReviewonBovine,SmallruminantandHumanBrucellosisinEthiopia.*Journalof Vet.Med.andResearch.*

Zinsstag,J.,Schelling,E.,Roth,F.,Bonfoh,B.,DeSavigny,D.andTanner,M.,2007.Humanbenefitsofanimalinterventionsforzoonosiscontrol;*Emerg.Inf.Dis*.**13**(4):527–553.

8.ANNEXES

Annex1:Datarecordingformatforbloodsampling

EpidemiologicalinvestigationofBrucellosisincattleofstudyarea

Zone_____District_____PA/Town_____village_____

S/No	Farmname	District	PA	Agro-ecology	HerdSize	Animalorigin	Age	Sex	Breed	Parity	MatingSystem	RPstatus	Historyofaborti	Frequencyofabortion	Gestationalstageofabortion	HistoryofRP,Stillbirth	Lab.result (+/-)	
																	RBPT	CFT
1																		
2																		
3																		
4																		
5																		

PA-PeasantAssociation;RP-Retainedplacenta(Yes/No);Historyofmaternalabortion(Y/N);RPStatus-ReproductiveStatus(Pregnant,Lactating,DrycowandHeifer);MatingSystem(naturalorAI);Originoftheanimals(born/bought).

Annex2:Questionnairesurveyfortheassessmentofbrucellosisandassociatedriskfactors.

Interviewintendedforlivestockowners/respondentsintheStudyarea

I.GeneralInformationondemographiccharacteristicsoftherespondents

Nameofrespondent_____Sex_____Zone_____

District_____PA/Town_____Mob.No_____

1.Occupationa.Governmentemployeeb.Non-governmentemployee(NGO)

c.Self-businessd.Farmer

2.Educationlevel?a.1-8gradeb.8-12gradec.>12grade

3.Locationa.urbanb.Peri-urbanc.rural

4.Areyoupracticingfarmanimalsastheonlywayoflife?a.Yesb.No

5.Ifyouhaveanotherjobspecifyjobtype……

6.Howdidyouacquireskillstoraisecattle/farming?

a)Agriculturaltraining(level)b)Fromextensionagents

c)Fromparentsd)other

II.Informationonherd(husbandryandmanagementsystem)

1.Herdtypeandsizeinyourfarm

Typeofcattle	Numberofanimals
Lactatingcows	
Pregnantcows	
Drycows	
Heifers	
Bull/ox	
Calves	

2.Whatisthefeedingmanagementofcattle?

a.Communalandfreegrazingb.Privateandfreegrazing

c.Tetherd.Stallfeed

3.Whichbreedofcattledoyouown?Whatisthenumberofcattleineachbreed?

Breedofcattle	Numberofcattle

Local	
25%cross	
50%cross	
Morethan50%	

4.Whattypeofbreedingsystemdoyouhaveforyouranimals?

a.AIb.NaturalMatingc.Both

7.Howisthehousingmanagement?

a)Barn(Separatelyormixedwithotherlivestock)

b)Corral(Separatelyormixedwithotherlivestock)

c)Openfield(Separatelyormixedwithotherlivestock)

d)Withinthefamilyhouse(Separatelyormixedwithotherlivestock)

e)Others

8.Whatareyourcullingcriteria?

a)Diseaseb)oldagec)infertilityd)poorproductione)other

9.Wheredoyougetyourreplacementstock?

……

10.Generalfarmhygiene/cleanliness

a)Verygoodb)Goodc)Satisfactoryd)Poor

III.Knowledge-attitudesandpracticesoffarmownersaboutbrucellosis

1.Haveyoueverseenreproductiveprobleminyourfarm?a.Yesb.No

2.Areyouawareofanydiseasethatcausesabortion?a.Yesb.No

Ifyes,whatisthelocalnamefordiseasethatcausesabortion?

……

3.Doyouknowaboutbrucellosis(“Gatachiisaa”inAfaanOromoo)?a.Yesb.No

4.Doyouthinkbrucellosisisazoonoticdisease?a.Yesb.No

5.Ifyesthroughwhichmeansdiseasecantransmit?

…….

6.Wasthereanyoccurrenceofabortion/stillbirthinyourfarm?Y/N

7.Ifyouranswerisyes,inwhichofthecowsandatwhichtimeofpregnancy?

Cowidentification	Timeofabortion
Heifer	
Cowatfirstcalving	
Cowatsecond	
Cowatthirdcalvingandmore	

8.Howmanyabortions/stillbirthsorretainedafterbirthhaveyouencounteredduringthelastthree years?a.Numberofabortion----------b.Numberofstillbirth----------

c.Numberofretainedfetalmembrane----------

9.Doyouseparatecowsduringparturition?a.Yesb.No

10.Doyouseparateabortedanimalfromother?a.Yesb.No

11.Doyoudisposeafterbirth?a.Yesb.No

12.Ifyeshowdoyoudisposeoftheafterbirth?

a.Burningb.Buryingc.Bothd.Throwntotheenvironment(opendump)

13.Istherefrequentcontactbetweenyourherdsandotheranimals?a.Yesb.No

14.Didyouseeanytesticularswelling?a.Yesb.No

15.Doyouconsumerawmilk?a.Yesb.No

16.Doyouboilmilk?a.Yesb.No

17.Doyouconsumerawmeatofcattle?a.Yesb.No

18.Doyouassistcowduringparturition?a.Yesb.No

19.Ifyouranswerisyes,doyouuseprotectivegloveduringassisting?a.Yesb.No

20.Whatdoyoudowiththeknown*Brucella*infectedanimals?

a.Separatetheinfectedanimalb.Selltoneighbor

c.Selltomarketd.taketothelocalveterinarianclinic

……

21.Haveyouintroducednewanimalsintoyourfarminthelastoneyear?Yes{}No{}Ifyes,howmanyCattle?

22.Didthefarm/herdbeentestedforbrucellosis?

…….....

Thankyouverymuchforparticipationandcooperationinthisstudy!!!

Theoutcomesofthestudywillbesharedamongstakeholderswheneveravailableforthepurposeofimprovinganimal,publicandenvironmentalhealth.Doyouhaveanycommentorquestionabouttheinterviewandourconversation?

_____

Annex3: Agedeterminationincattlebasedonteetheruption

No	Teeth	Ages
1	I1erupts	11/2-2years
2	I2erupts	2-21/2years
3	I3erupts	3years
4	Cerupts	3½-4years

5	Allincisorsarewear	5years
6	I1islevelandtheneckhasemergedfromthegum	6years
7	I2islevelandtheneckisvisible	7years
8	I3isleveltheneckisvisible	8years
9	Cislevelandtheneckisvisible	9years
10	Theteeththathavenotfallenoutarereducedtosmallroundpegs	15years

Source:PaceandWakeman,1983

Annex4:RoseBengalPlateTest(RBPT)reagents,materialandequipmentandprocedure(OIE,2016).

ReagentsandmaterialsrequiredforRBT

Reagents:

- RoseBengalTest*Brucella*antigen
- Positivecontrolsera(frompreviouslypositiveserum)
- Negativecontrolsera(frompreviouslynegativeserum)

- Testsera

Materials

- Plate
- Micropipetteof30µl
- Micropipettetips
- Applicator
- Tubeofserumcollection
- Magnifyingglass
- Vacutainertubesfittedwithhandleandneedles
- Rack

Procedures

Thetestseraandtheantigenwillbeleftataroomtemperatureforhalfanhoureverytimebeforethetestisproceeded.

- 30microlitterofeachtestserumwillbetakenandplacedonacleanglassslide.
- 30microliterofRBPTantigenwillbeaddedtothesideofeachtestserumusingepindorftube.
- Thentheantigenandthetestserumweremixedthoroughlybyanapplicator
- Theglassslidewasshakedbyhandfor4minutesand
- Finallytheresultofeachtestwasreadbylookingthepresenceorabsenceofagglutinationandthedegreeofagglutinationwasalsoappreciatedinaverygoodlightsourceandwhennecessarymagnifyingglasseswereused.

Interpretation: Afterfourminutesrocking(shaking)anyvisibleagglutinationwasconsideredpositive.

Annex5: CompetitiveELISAtestprocedures(COMPELISA160and400,NewHaw,Addlestone,andSurrey,KT153NB,UnitedKingdom).

1. Warmthedilutingbuffertoroomtemperature,itisrecommendedthatthedilutingbufferiswarmedinawaterbathat23ºC(±3ºC).Mixtheconjugateconcentrate(BM40)thoroughlyanddilutetoworkingstrengthinthewarmeddiluting.
2. Add20µlofeachtestserumperwell.Leavecolumnsforcontrols.
3. Add20µlofthepositivecontroltowellsforstrongpositiveandweak.

4. Add20µlofthenegativecontroltowellsfornegatives.
5. Then,100µlofpreparedconjugateSolutionwasaddedtoeachwell
6. Theplateisthenvigorouslyshaken(onthemicrotitreplateshaker)fortwominutesinordertomixtheserumandconjugatesolution.Covertheplatewithalidandincubateatroomtemperature(21°C±6°C)for30minutesonarotaryshaker,at160revs/min.
7. Shakeoutthecontentsoftheplatebeforewashingeachplate5timeswitheitherwashingsolutionordrinkingwaterfromatapunderlowpressure(keeptapwateratasteady,softflow).Dryplatebytappingfirmlyontoafewlayersofabsorbenttoweluntilnomoreliquidisremoved
8. Add100µlofsubstratesolutiontoallwellsusingamulti-channelpippeteandincubatetheplateatroomtemperature(21°C±6°C)foraminimumof10minutesandamaximumof20minutes.
9. Switchonmicroplatereaderandallowtheunittostabilizefor10minutes
10. Slowthereactionbyadding100µlofstoppingsolutiontoallwells.
11. Lastly,theOpticalDensity(OD)ofthecontrolsandsampleswasmeasuredat450nminaMicroplatephotometer(Flowlaboratories,UK)immediatelyafteradditionofStopsolutiontopreventfluctuationsinODvalues.Thelackofcolordevelopmentindicatesthatthesampletestedwaspositive. Apositive/negativecut-offcanbecalculatedas60%ofthemeanoftheopticaldensity(OD)ofthe4conjugatecontrolwells. AnytestsamplegivinganODequaltoorbelowthisvalueshouldberegardedasbeingpositive.