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Mathematical Modeling and Control of Brucellosis in El Oued Province, Algeria

Submitted by

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Abstract

Brucellosis is an endemic zoonotic disease responsible for enormous losses in animal industry and public health in Algeria. Therefore, designing a control/eradication policy adjusted to epidemiological and socioeconomic conditions appeared to be a requisite. In this context, the present PhD project aimed to simulate different control strategies over 20 years in order to predict the optimum approach to eradicate the brucellosis in small ruminant in El Oued district. Therefore, a cross sectional study was carried out among small ruminant flocks to determine the herd and individual true prevalence. Six hundred and twelve (612) sera samples were screened for anti-*Brucella* spp. antibodies using Rose Bengal test (RBT), and indirect enzyme-linked immunosorbent assay (iELISA) in parallel and complement fixation test (CFT) for confirmation. Afterward, a deterministic mathematical model of the dynamic spread of brucellosis in small ruminant using compartmental model and a deterministic simulation agent-based-model (ABM) of different control strategies were developed. Indispensable data in relation to *Brucella melitensis*, characteristics of animal population in the study area, vaccination, and the performance of serological tests were collected meticulously. True herd prevalence was 27.95% (95% CI: 17.18–42.01) and true individual prevalence was 3.98% (95% CI: 2.51–6.03). Sampling of 50% of adults to be culled and slaughtered after positive reaction to RBT and CFT used in serial testing revealed to be the optimum strategy to eradicate the disease for about 18-19 years in the study area. Combining vaccination of young animals may enhance slightly the effectiveness of the eradication policy, but would not be cost-effective for a long-term program. Based on our findings, the adoption of test-and- slaughter strategy in order to eliminate the disease in El Oued district is strongly advised. However, cooperation and willingness of all stakeholders is vital for the adopted program to be effective and fruitful. Similar studies in distinct ecological areas and unified epidemiological conditions are highly recommended to draw up an optimal control/eradication plan.

Key-words: El Oued, Brucellosis, Control-strategies, Cross-sectional study, Modeling, Simulation, Small ruminant

Résumé

La brucellose est une zoonose endémique responsable d'énormes pertes en filière animale et en santé publique en Algérie. La conception d'un plan de lutte/éradication adaptée aux conditions épidémiologiques et socio-économiques semblait indispensable. Dans ce contexte, ce projet de thèse visait à simuler différentes stratégies de lutte dans les prochaines 20 ans afin de prédire la stratégie optimale pour éradiquer la brucellose des petits ruminants dans la wilaya d'El Oued. En effet, une étude transversale de séroprévalence chez les petits ruminants a été réalisée pour déterminer la prévalence réelle individuelle et du cheptel. Six cent douze (612) échantillons de sérums ont été testés pour détecter les anticorps anti-*Brucella* spp. par l'usage du test Rose Bengale (RBT) et de la technique immuno-enzymatique indirecte (iELISA) en parallèle et le test de fixation du complément (CFT) pour la confirmation. Ensuite, un modèle mathématique déterministe de la dynamique de transmission de la brucellose des petits ruminants en utilisant un modèle à compartiments et un modèle déterministe de simulation à base d'individus de différentes stratégies de contrôle ont été développés. Les données indispensables concernant *Brucella melitensis*, les caractéristiques de la population animale dans la zone d'étude, la vaccination et les performances des tests sérologiques ont été minutieusement recueillies. La prévalence réelle du troupeau était de 27,95 % (IC à 95 % : 17,18 à 42,01) et la prévalence individuelle réelle était de 3,98 % (IC à 95 % : 2,51 à 6,03). L'échantillonnage de 50 % des adultes à assainir suite à une réaction positive aux RBT et CFT utilisés en série s'est révélé être la stratégie optimale pour éradiquer la maladie dans la zone d'étude dans environ 18-19 ans. La combinaison de la vaccination des jeunes animaux pourrait améliorer légèrement l'efficacité de la politique d'éradication, mais ne serait pas rentable pour un programme à long terme. Sur la base de nos conclusions, l'adoption d'une stratégie de test et d'abattage afin d'éliminer la maladie dans la wilaya d'El Oued est fortement conseillée. Cependant, la coopération et la volonté de toutes les parties prenantes sont essentielles pour que le programme adopté soit efficace et fructueux. Des études similaires dans des zones écologiques distinctes et des conditions épidémiologiques unifiées sont fortement recommandées pour élaborer un plan de lutte/éradication optimal.

Mots-clés : El Oued, Brucellose, Lutte-stratégies, Etude transversale, Modélisation, Simulation, Petits ruminants.

الملخص

تعتبر الحمى المالطية من الأمراض الحيوانية المنشأ والمستوطنة في الجزائر، حيث تسبب هذا المرض بخسائر فادحة في الإنتاج الحيواني والصحة العمومية. و لهذا، تصميم برنامج لمكافحة و التخلص من هذا المرض يتلاءم مع الظروف الوبائية، الاجتماعية و الاقتصادية يعد أمر ضروري. في هذا الصدد، يهدف مشروع الدكتوراه الحالي إلى محاكاة الاستراتيجيات المختلفة لمكافحة داء البروسيلا لدى المجترات الصغيرة في منطقة الوادي على مدى 20 عامًا من أجل التنبؤ بالاستراتيجية الأكثر فعالية. لذلك، تم إجراء دراسة مقطعية بين قطعان المجترات الصغيرة لتحديد نسبة الانتشار الحقيقي للقطيع والفرد. حيث تم فحص ستمائة واثني عشر (612) عينة مصل للبحث عن الأجسام المضادة للبروسيلا باستخدام اختبار Rose Bengal (RBT) و اختبار iELISA على كل العينات. وتم التأكد من العينات الإيجابية باستخدام اختبار Complement fixation test (CFT). بعد ذلك، تم تطوير نموذج رياضي حتمي للانتشار الديناميكي لداء البروسيلا في المجترات الصغيرة باستخدام نموذج مجزأ ونموذج قائم على المحاكاة الحتمية (ABM) لاستراتيجيات مختلفة لمراقبة المرض. لهذا الهدف، تم جمع البيانات المهمة بدقة و التي تخص بكتيريا البروسيلا المالطية *Brucella melitensis*، خصائص الحيوانات في منطقة الدراسة ، التحصين ضد مرض الحمى المالطية، وأداء الاختبارات المصلية. قدر الانتشار الحقيقي للقطيع بـــ 27.95٪ (مجال الموثوقية 95٪: 17.18-42.01) كما قدر الانتشار الفردي الحقيقي بـــ 3.98٪ (مجال الموثوقية 95٪: 2.51-6.03). أظهر أخذ عينات من 50٪ من البالغين المراد ذبحهم بعد التفاعل الإيجابي لـ RBT و CFT المستخدمة بطريقة تسلسلية أنها الاستراتيجية المثلى للقضاء على المرض لمدة 18-19 عامًا في منطقة الدراسة. قد يؤدي الجمع بين تحصين صغار الحيوانات إلى زيادة طفيفة في فعالية سياسة الاستئصال ولكنه لن سيكون مكلفًا للغاية لبرنامج طويل المدى. بناءً على النتائج التي توصلنا إليها، يُنصح بشدة باعتماد استراتيجية الاختبار والذبح من أجل القضاء على المرض في منطقة الوادي. ومع ذلك، فإن تعاون واستعداد جميع الأطراف المعنية يعتبر أمر أساسي لكي يكون البرنامج المعتمد فعالاً. كما يوصى بشدة إجراء دراسات مماثلة في مناطق بيئية مختلفة وظروف وبائية موحدة لوضع خطة مكافحة / استئصال مثالية.

الكلمات المفتاحية: الوادي، الحمى المالطية، استراتيجيات التحكم ، دراسة مقطعية ، النمذجة ، المحاكاة ، المجترات الصغيرة.

Table of Contents

List of tables

List of figures

List of abbreviations and symbols

OIE : International Office of Epizootics OIE historical acronym of World Organization for Animal Health WOAH (OMSA in French)
INSP : French acronym of the National Institute of Public Health (In French: Institut National de Santé Publique)
MADR: French acronym of the Algerian Ministry of Agriculture and Rural Development (Ministère d'Agriculture et du Développement Rural)
WHO : World Health Organization
WOAH : World Organization for Animal Health
WAHIS : World Animal Health Information System
Bv: Biovar
LVR : French acronym of the Regional Veterinary Laboratory
RBT : Rose Bengal Test
iELISA: Indirect Enzyme-Linked Immunosorbent Assay
DALYs: Disability-Adjusted Life Years
INR : Indian rupee (Currency of India)
Peseta: Previous currency of Spain
DA : Algerian Dinar (Currency of Algeria)
EUR : Euro (the official currency of 19 out of the 27 member states of the European Union)
DNA : Deoxyribo-Nucleic Acid
MLVA: Multiple-Locus Variable analysis
MLST: Multi-Locus Sequence Typing
SNP : Single Nucleotide Polymorphism
LPS: Lipopolysaccharide
S-LPS : Smooth Lipopolysaccharide
R-LPS: Rough Lipopolysaccharide
OMP : Outer Membrane Proteins
OPS : O-chain of Polysaccharide
CP28: Cytosoluble Protein 28
BP26 : Binding Protein 26
GroES : Chaperonin protein
DnaK : Chaperonin protein
MHC-II: Major Histocompatibility Complex class II
IL-12 : Interleukin 12
PCR : Polymerase Chain Reaction
MLSA : Multi-Locus Sequence Analysis
BBAT : Buffered *Brucella* Antigen Tests
BPAT: Buffered Antigen Plate Agglutination Test
CFT : Complement Fixation Test
MRT : Milk Ring Test
FPA : Fluorescence Polarization Assay
AGID : Agar Gel Immuno-Diffusion
RID : Radial Immuno-Diffusion
R0 : Basic Reproduction Number
DFE: Disease-Free Equilibrium
HN: Herd Number
HTP: True Herd Prevalence
HSENS: Herd Sensitivity
HSPEC: Herd Specificity

C: Confidence limits
Z: Z-score, or Z-statistic
L: Absolute precision
se: Sensitivity
sp: Specificity
TP: True individual Prevalence
AP: Apparent Prevalence
HAP: Herd Apparent Prevalence
N : Total number of small ruminant
S : Susceptible
I : Infected
V : Vaccinated
C: Slaughtered animals
B : Birth rate
μ : Death rate
$\alpha_{1:}$ Young animals of replacement rate
ß : Transmission rate
ODE: Ordinary Differential Equations
R(t) : Effective reproduction number
DEE : Disease Endemic Equilibrium
ɣ : Proportion of vaccinated animals
ε : Efficacy of Rev1 vaccine
σ : Waning immunity rate of vaccine
α_2: Proportion of adult animals non-infected and non-vaccinated
κ : Proportion of animals to be sampled
Se_b : Sensitivity of RBT and CFT in series
Sp_b : Specificity of RBT and CFT in series
$\frac{ds}{dt}$: Derivative of susceptible at time t
$\frac{di}{dt}$: Derivative of infected at time t
$\frac{dv}{dt}$: Derivative of vaccinated at time t
Tmax : Maximum Time (20 years)
dt: Time step

General Introduction & Thesis Outline

Brucellosis is a bacterial zoonotic, caused by several species of the genus *Brucella*. The most prominent species in terms of their effects on both animal and human disease are *B. melitensis, B. abortus,* and *B. suis* (Whatmore et al., 2016). *B. melitensis* is the most prevalent and pathogenic species causing human brucellosis (Alton and Forsyth, 1996; Corbel, 2006). The disease has wide-ranging and detrimental consequences on both humans and animals in endemic areas (Franc et al., 2018). Brucellosis is prevalent worldwide. However, it is endemic in the Middle East, the Mediterranean region, Sub-Saharan Africa, China, India, Peru, Mexico, central and southwest Asia (OIE, 2022a).

Algeria has implemented a number of control measures against bovine brucellosis since 1970. Goats were incorporated into a test-and-slaughter program from 1995 to 2006 with cattle. However, from 2006 to 2017, vaccination of young small ruminants with Rev1 vaccine was gradually added to the prior policy (MADR 2021). Nonetheless, Brucellosis is still endemic in Algeria causing more than 10 000 human cases (INSP, 2017). Moreover, the country is reported to be one of the mediterranean countries with the highest incidence of brucellosis (OIE, 2022a). Control/eradication programs including three policies that consist of mass vaccination, test-and-slaughter and vaccination of young small ruminant were suggested (Benkirane, 2006; Minas, 2006; Blasco, 2010). However, relevant factors including the prevalence and other epidemiological and socio-economic factors should be taken into account to plan the optimum control strategy for each area (Pérez-Sancho et al., 2015). On the other hand, using mathematical modeling in epidemiology has served as an intelligent and economical tool to simulate various control programs and predict their outcome over time. Modeling of brucellosis and simulation of control approaches over 20 years in order to design an optimal policy was the main objective of our PhD project.

Minas (2006) and Blasco (2010) stated that each ecologically distinct area depending on the epidemiological and socio-economic aspects requires a unique policy for control or eradication of small ruminant brucellosis. From this point, we have carried out two studies in El Oued district which is situated in the southeast of Algeria:

1. A cross sectional study in order to determine the prevalence of brucellosis in small ruminant herds and provide further understanding regarding the epidemiological context of the disease in this specific study area.
2. Modeling of the dynamic of the transmission of brucellosis in small ruminant and simulation of various proposed control strategies over 20 years.

This dissertation is organized into two main parts:

1) The first part consists of literature review. The first chapter discusses brucellosis in general in the world, Algeria and in El Oued district; its impact on the animal health and production and on the public health and economy; the agent pathogen characteristics and review of brucellosis in small ruminant. The second chapter addresses modeling of infectious diseases in epidemiology; describing its history and definition, advantages and classification of modeling, epidemic and endemic models and equilibrium sates.
2) The second part provides details of the two conducted studies: the cross- sectional study of small ruminant brucellosis and modeling of brucellosis in small ruminant in El Oued district, respectively, describing the problem and objectives, materials and methods, results, discussion and conclusion of each study apart.
3) At the end of this dissertation, the main conclusions and recommendations are outlined. The general discussion summarizes the background, problems and the major findings of the present research project. It also manifests the relevance, pitfalls and limitations of the project.

Literature Review

Chapter 1

Review of brucellosis

1. Brucellosis in the past and present

Brucellosis was recognized the first time in the 1850s in Malta when the British servicemen that served in the Mediterranean after "the Crimean War" contracted a fever called ‘Malta’, ‘Gibraltar’ or ‘Cretan fever’ (Wyatt, 2013). The bacterium was firstly isolated in 1887 by Sir David Bruce and Lady Bruce, together with Dr. Guiseppe Caruana Scicluna (Wyatt, 2013).

Brucellosis is widespread worldwide (WHO, 2022). According to the World Health Organization, 500 000 cases reported yearly. However, it is classified as one of the seven most neglected diseases (Pappas et al., 2010).

In general, the Middle East, the Mediterranean region, sub-Saharan Africa, China, India, Peru, and Mexico reported the highest incidence (OIE, 2022a). According to the World Organisation for Animal Health (WOAH), a notable increase is observed currently in central and southwest Asia countries. Whereas, multiple countries such as Western and Northern Europe, Canada, Japan, Australia and New Zealand are declared to be free of *Brucella* (OIE, 2022a).

Based on the data collected in the year 2021 from the World Animal Health Information Database Interface (WAHIS; http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home) (Table I), Saudi Arabia recorded the highest incidence of *B. melitensis* infection in small ruminant by over 5000 cases, followed by Armenia by over 2 000 cases, then Italy by over 1000 incident cases. Whereas, several countries reported the presence of *B. melitensis* infection in other species such the case in Saudi Arabia by detecting about 200 incident cases in Camels. Armenia recorded also more than 100 cases in Cattle. Whereas, Russia, Armenia, Italy, Paraguay, Brazil and South Africa recorded more than 2000 cases of *B. abortus* infection in Cattle and Buffaloes. Other countries reported high incidence of *B. abortus* infection in Cattle, among others, Costa Rica, Iran and Mexico (over 1000 cases). Brucellosis infection due to *B. suis* in suidae was reported in some countries. Spain, Germany, Romania, France, Uruguay and Italy recorded incident cases between 7 and 57 in the year 2021.However, according to Hull and Schumaker (2018), this interface of WAHIS has the disadvantage of over and underestimation of records due to voluntary reporting and the competence of surveillance systems.

Table I: Number of cases of animal brucellosis reported in 2021 at the WOAH (WAHIS platform)

Year	Continent	Country	Agent	Species	Cases
2021	Asia	Arabia Saudi	*Brucella melitensis*	Sheep and Goats	5 213
2021	Europe	Russia	*Brucella abortus*	Cattle	3 545
2021	Asia	Armenia	*Brucella abortus*	Cattle	3 236
2021	Europe	Italy	*Brucella abortus*	Buffaloes	2 561
2021	America	Paraguay	*Brucella abortus*	Cattle	2 435
2021	America	Brazil	*Brucella abortus*	Cattle	2 426
2021	Africa	South Africa	*Brucella abortus*	Cattle	2 383
2021	Asia	Armenia	*Brucella melitensis*	Sheep and Goats	2 058
2021	America	Costa Rica	*Brucella abortus*	Cattle	1 641
2021	Asia	Iran	*Brucella abortus*	Cattle	1 390
2021	Europe	Italy	*Brucella melitensis*	Sheep and Goats	1 248
2021	Europe	Italy	*Brucella abortus*	Cattle	1 028
2021	America	Mexico	*Brucella abortus*	Cattle	1 027
2021	Asia	Pakistan	*Brucella abortus*	Cattle	998
2021	America	Ecuador	*Brucella abortus*	Cattle	964
2021	Asia	Iraq	*Brucella melitensis*	Sheep and Goats	811
2021	Asia	Azerbaijan	*Brucella abortus*	Cattle	439
2021	America	Mexico	*Brucella melitensis*	Goats	392
2021	Asia	Iran	*Brucella melitensis*	Sheep and Goats	371
2021	Asia	Kuwait	*Brucella melitensis*	Sheep and Goats	322
2021	Europe	Russia	*Brucella melitensis*	Sheep and Goats	297
2021	Asia	Thailand	*Brucella melitensis*	Sheep and Goats	249
2021	America	Uruguay	*Brucella abortus*	Cattle	206
2021	Asia	Arabia Saudi	*Brucella melitensis*	Camel	188
2021	Asia	Azerbaijan	*Brucella melitensis*	Sheep and Goats	185
2021	Asia	Israel	*Brucella melitensis*	Sheep and Goats	124
2021	Asia	Armenia	*Brucella melitensis*	Cattle	122
2021	Asia	Georgia	*Brucella abortus*	Cattle	101
2021	America	Costa Rica	*Brucella abortus*	Buffaloes	97
2021	Asia	Israel	*Brucella melitensis*	Cattle	85
2021	America	Nicaragua	*Brucella abortus*	Cattle	64
2021	Europe	Italy	*Brucella suis*	Suidae	57
2021	Asia	Pakistan	*Brucella abortus*	Buffaloes	56
2021	Europe	Spain	*Brucella melitensis*	Sheep	54
2021	Asia	Yemen	*Brucella melitensis*	Sheep and Goats	42
2021	America	Uruguay	*Brucella suis*	Suidae	35
2021	Asia	Malaysia	*Brucella abortus*	Cattle	33
2021	Europe	France	*Brucella suis*	Suidae	30
2021	Europe	Romania	*Brucella suis*	Suidae	30

Table I: Number of cases of animal brucellosis reported in 2021 at the WOAH (WAHIS platform) (Continued)

2021	Africa	South Africa	*Brucella abortus*	African buffalo	28
2021	Asia	Syria	*Brucella melitensis*	Sheep and Goats	20
2021	Asia	United Arab Emirates	*Brucella melitensis*	Sheep and Goats	16
2021	Europe	Germany	*Brucella suis*	Suidae	13
2021	Asia	Yemen	*Brucella abortus*	Cattle	12
2021	Africa	Djibouti	*Brucella melitensis*	Goats	11
2021	Asia	Iraq	*Brucella abortus*	Cattle	10
2021	Africa	Somalia	*Brucella abortus*	Cattle	8
2021	Africa	Somalia	*Brucella melitensis*	Sheep and Goats	8
2021	America	Peru	*Brucella abortus*	Cattle	7
2021	Asia	Thailand	*Brucella abortus*	Cattle	7
2021	Europe	Spain	*Brucella suis*	Suidae	7
2021	Africa	Djibouti	*Brucella abortus*	Cattle	6
2021	Asia	Kuwait	*Brucella melitensis*	Cattle	6
2021	Asia	Syria	*Brucella abortus*	Cattle	5
2021	Asia	Malaysia	*Brucella melitensis*	Sheep and Goats	3
2021	Africa	Nigeria	*Brucella abortus*	Cattle	2
2021	Africa	Nigeria	*Brucella abortus*	Sheep	2
2021	Asia	Arabia Saud	*Brucella melitensis*	Cattle	2
2021	America	Mexico	*Brucella melitensis*	Cattle	1
2021	America	Peru	*Brucella melitensis*	Goats	1
2021	Asia	Iraq	*Brucella abortus*	Buffaloes	1
2021	Asia	Thailand	*Brucella abortus*	Buffaloes	1
2021	Europe	France	*Brucella melitensis*	Cattle	1

2. Brucellosis in Algeria

Algeria is an endemic country regarding brucellosis. Based on the report of National Institute of Public Health (INSP, 2017), 10 198 human incident cases of brucellosis were notified. Djelfa, M'slia, Laghouat and Tebessa recorded the highest incidence with more than 1000 cases. El Bayadh, Naama, Bechar and Biskra reported more than 300 cases. Over 100 cases were reported in Oum Bouaghi, Batna, Tlemcen, Tiaret, Setif, Sidi Bel Abbes, Tindouf, El Oued and Khenchela. The remaining provinces reported less than 100 cases (Figure 1).

B. abortus biovars (bv) 3, *B. melitensis* bv 2 and *B. melitensis* bv 3 were identified in cattle in different regions of Algeria (Lounes et al., 2021). *B. melitensis* bv 3 is mostly the causative agent of human and small ruminant infection in Algeria (Gabli et al., 2015; Lounes et al., 2021).

Studies conducted in the past recent years obtained varied seroprevalences in different species by using various methods. Kardjadj et al. (2016) found 3.33 % of seropositive small ruminant flocks countrywide. Yahia et al. (2020) found 1.4 % of *Brucella* seropositivity in cattle in Djelfa. Kaaboub et al. (2019) found 2.5 % of seropositive cattle sampled from the slaughterhouse in Medea province.

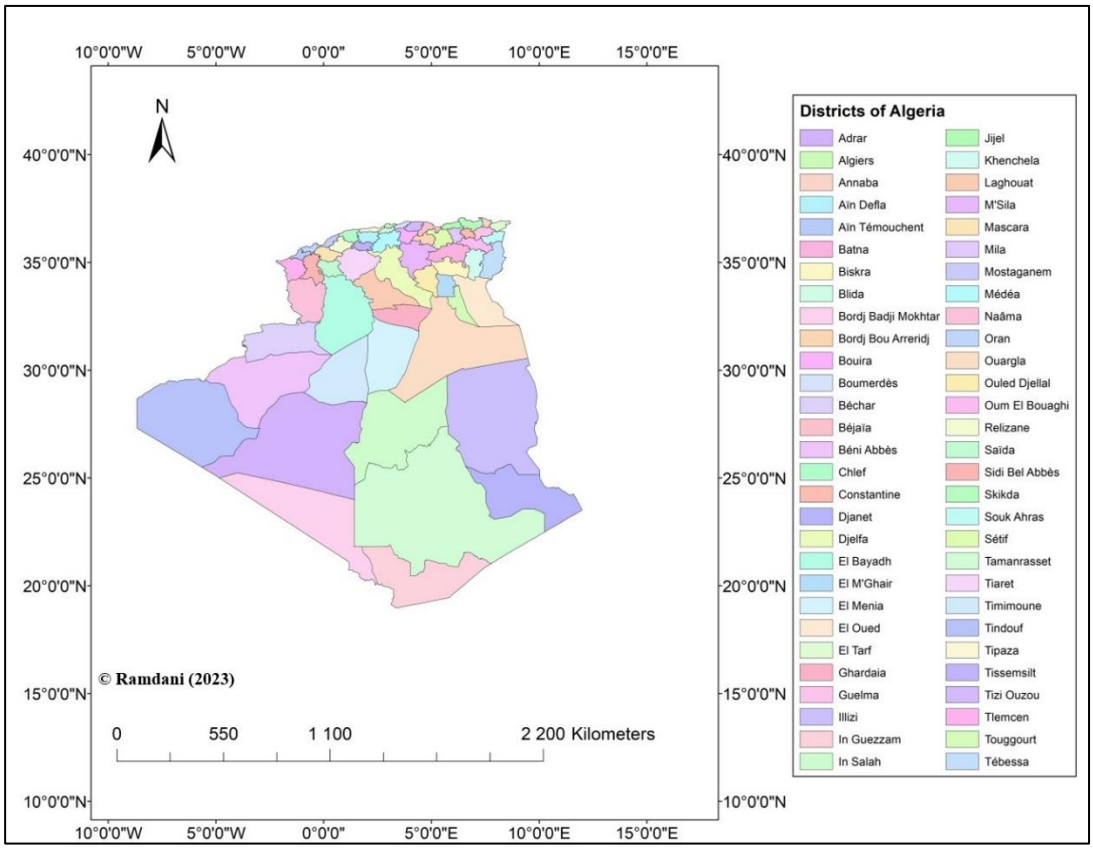

Figure 1: Map of administrative division of Algeria

3. Brucellosis in El Oued

According to the Directorate of Health and Population of El Oued province (DSP El Oued, 2022), 110 human cases were reported during the year of 2021. Most cases were recorded in Ben Guecha (25 cases), followed by Hassi Khalifa (23 cases), Sidi Aoun (12 cases) and Taleb Larbi (10 cases) (Figure 2). However, only 09 cases of seropositive goats were reported in 2021 located at El Oued and Robbah municipalities (Figure 2) by the Regional Veterinary Laboratory of El Oued (LVR El Oued, 2022).

Nevertheless, these figures are supposed to be the source of human infection. Moreover, one infected animal can transmit the disease to dozens of people. Furthermore, this may also imply that animal brucellosis is even more underestimated or under-reported than human brucellosis in this region.

A cross-sectional study conducted previously by Ramdani and Ghalmi (2017) on goat family farm at El Oued province revealed 8.67% and 2.04% of seropositive goats by using RBT and iELISA, respectively.

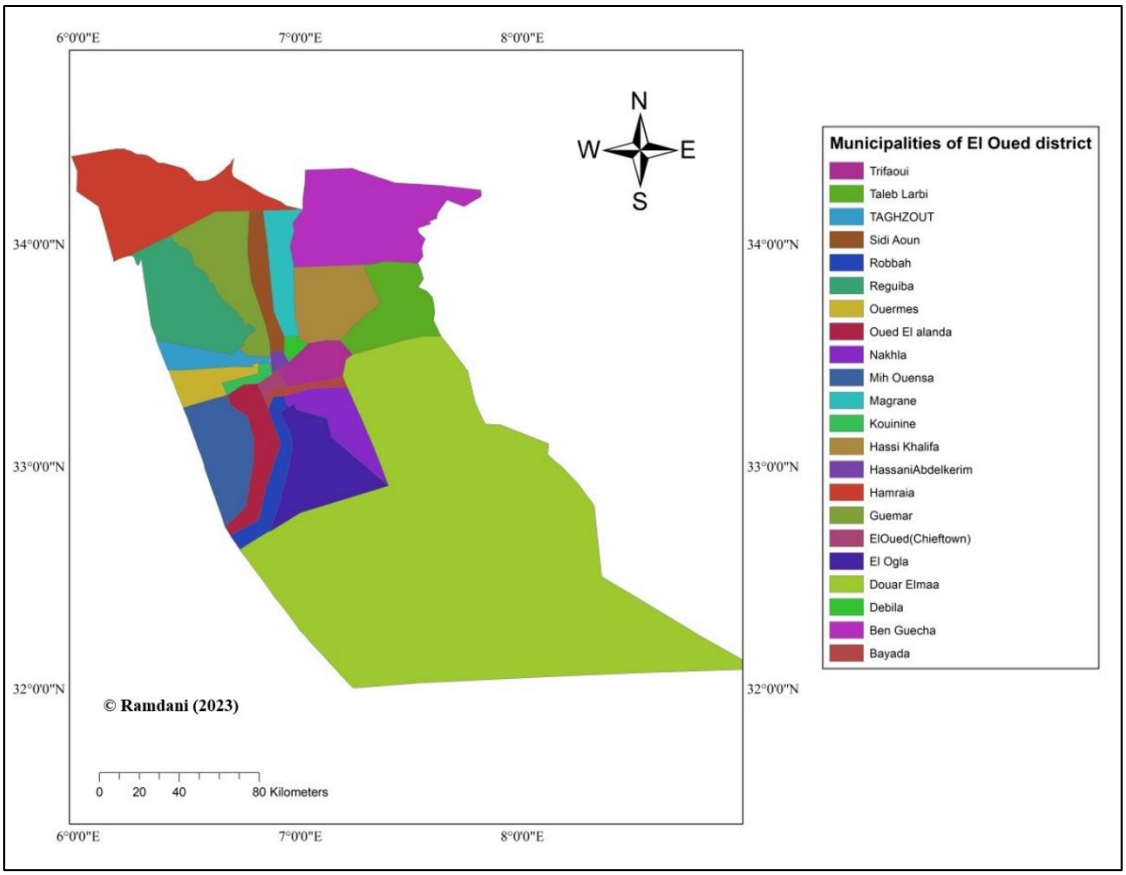

Figure 2: Map of administrative division of El Oued district

4. Impact of brucellosis

4.1. Animal brucellosis impact

According to Rushton et al. (1999), the impact of animal brucellosis can be direct or indirect. The direct effect appears through losses due to abortions in farms, drop in milk production, loss of weight gain due to chronic infections, loss of traction power, stillbirths, culling of infected animals and decrease of animal welfare. Indeed, several studies carried out in Pakistan, India and Ethiopia have revealed a significant association ($p<0.05$) of abortion history with *Brucella* spp. seropositivity (Saeed et al., 2019; Behera et al., 2020; Edao et al., 2020; Shakeel et al., 2020). Additionally, in Mexico, a study was conducted in a dairy farm where the application of brucellosis control program for a period of six years, led to a rise in annual milk production by a percentage of 21% (Herrera et al., 2008). Moreover, Tadeg et al. (2015) reported that weak-born offspring prone to early mortality were significantly associated with brucellosis seropositivity ($p<0.05$). Furthermore, brucellosis revealed to be responsible for elimination of 34–62% in dairy farms due mainly to infertility and abortion (Herrera et al., 2008). Frank et al. (2018) highlighted in infected animals, the loss of draught power used for transport and/or traction and the occurrence of carpal hygromas, the most common clinical sign of brucellosis in cattle, which causes inflammation, joint pain and reduced mobility. In addition, Singh et al. (2015) estimated brucellosis losses in Indian livestock (cattle, buffaloes, sheep, goats and suidae) by USD $3.4 billion evaluated by production drop, reduced fertility and mortalities. In Brazil, losses caused by bovine brucellosis that were assessed by abortion and perinatal mortality rates, temporary infertility, replacement costs, mortality, veterinary costs, milk and meat losses were estimated to be approximately USD $448 million (Santos et al., 2013). The same authors reported that the economic impact of brucellosis is anticipated to change by 155 million Reais for every 1% rise or decrease in prevalence.

The indirect effects of brucellosis may be summed up in losses due to limitation of trade and access to international markets, the costs of vaccination campaigns and control programs and the costs of implementing veterinary infrastructure (Rushton et al., 1999). In fact, according to the guidelines of the terrestrial animal health code of the World Organization for Animal Health (OIE, 2019), the movement or transport of potentially infected animals or animal products to zones or countries free of the Brucellosis is prohibited or authorized for importation under certain conditions that its application is challenging.

Moreover, brucellosis eradication program in the United States cost approximately USD $3.5 billion between 1934 and 1997 (Sriranganathan et al., 2009). Whereas, in Kazakhstan, Charypkhan et al. (2019) estimated the costs of compensation for animals slaughtered for reasons of brucellosis seropositivity by 21 million dollars, and the costs of animal analyzes by 24 million during 2015.

4.2. Human brucellosis impact

Human brucellosis is classified as an acute, sub-acute or chronic febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia, and arthralgia (Doganay and Aygen 2003; Corbel, 2006). Human brucellosis is acquired mainly through consumption of contaminated raw milk and unpasteurized dairy products, contact with infected animals, and inhalation of contaminated aerosols (OIE, 2022b). Three species are the most pathogenic for humans: *B. melitensis*, *B. abortus* and, *B. suis*. *B. melitensis* is the most invasive and virulent species for humans (Alton and Forsyth, 1996; Corbel, 2006). It was also found to be the most commonly discovered cause of human brucellosis in endemic areas of the world and a significant foodborne pathogen in underdeveloped nations (Georgi et al., 2017; Bagheri Nejad, et al., 2020).

As defined by Jo (2014), the impact of human brucellosis is mostly evaluated by DALYs (disability-adjusted life years), which account for medical expenses as well as "missed economic or societal contribution" brought on by early death or disability.

According to Jo (2014), the costs are classified into three categories: direct, indirect and intangible costs. Intangible costs are those that lower a patient's quality of life but cannot be effectively standardized amongst people and, thus, often have no known monetary value (Jo, 2014; Franc et al., 2018). However, healthcare costs for diagnosing, treating, and managing patients who are clinically ill are examples of direct costs, whereas the costs of a disease's morbidity and death that directly impact the patient and the community in which they reside are known as indirect costs (Jo, 2014; Franc et al., 2018).

In Kazakhstan, 1334 human cases were reported in 2015 resulting in 713 DALYs (disability-adjusted life years) (Charypkhan et al., 2019).

Human brucellosis in India caused 627.5 million INR of the annual median losses and 177 601 DALYs (Disability Adjusted Life Years) at the rate of 0.15 DALYs per thousand persons per year (Singh et al., 2018).

In Spain, the burden of human brucellosis was estimated in 1986 to be 787.920 pesetas per patient including 287.149 pesetas for a hospitalization of 13 days and absence from work for 102 days (Colmenero Castillo et al., 1989).

Benhabyles et al. (1992) reported that brucellosis in Algeria cost about 12 000 DA per patient in 1990 which was equivalent of 8 months of minimum wage per patient suffering from acute septicaemic disease that requires hospitalization of 7 days and 45 days home care. According to the same authors, this estimation included the cost of hospital stay (hotel), laboratory testing and treatment.

Akakpo et al. (2009) reported that the cost of treatment per patient in Africa was estimated by 9 EUR in Tanzania, 200 EUR in Morocco, and 650 EUR in Algeria.

5. Pathogen agent

5.1. Classification and characterization

Brucella spp. are gram-negative bacteria, coccoid or short rod-shaped cells from 0.5-0.7x 0.6-1.5 microns in size, belonging to the family *Brucellaceae* (Banai and Corbel, 2010). *Brucella* spp. are non-motile, non-sporing, aerobic, facultative intracellular pathogens of the reticulo-endothelial cells of terrestrial and marine mammal hosts (Banai and Corbel, 2010; Percin, 2013).

The discovery of new *Brucella* in recent years has greatly expanded the genus, which currently comprises 12 recognized species, three of which, namely *B. melitensis*, *B. abortus* and *B. suis* are the main causes of brucellosis in humans (Alton and Forsyth, 1996; Whatmore et al., 2016).

Brucella species were identified based on specific host preferences, phage susceptibility, and oxidative metabolism patterns with certain carbohydrate and amino acid substrates (Banai and Corbel, 2010). Despite the strong DNA homologies of all species and subspecies of *Brucella* genus, small variations between the species have been validated by whole genome analysis like MLVA (Multiple-Locus Variable analysis), MLST (Multi-locus sequence typing), microarray studies and SNP (single nucleotide polymorphism) (Banai and Corbel, 2010).

By separating the six classical species (*B. abortus, B. melitensis, B. suis*, *B. ovis, B. canis* and *B. neotomae*) and further subdividing the three major species into seven (*B. abortus*), five (*B.* suis), and three (*B. melitensis*) biovars, respectively, a biotyping scheme based on a combination of growth characteristics, biochemical reactions, serotyping, and bacteriophage typing is used (Whatmore et al., 2016). Additionally, based on lipopolysaccharide (LPS) expression, *Brucellae* are separated into two categories: rough and smooth. Smooth species that fully express the O-chain have higher virulence than rough species that express little to no O-chain (Lapaque et al., 2005).

Smooth species are composed of *B. abortus, B. melitensis, B. suis, B. neotomae, B. ceti,* and *B. pinnipedialis, B. microti*, B. *inopinata, B. papionis* (Muñoz et al., 2022). *B. canis* and *B. ovis,* are rough species (Muñoz et al., 2022).

5.2. Antigenic structure

Within the genus *Brucella*, the following immunodominant antigens have been discovered: (1) smooth lipopolysaccharide (S-LPS), (2) rough lipopolysaccharide (R-LPS), (3) outer membrane proteins (OMP), and (4) periplasmic and cytoplasmic proteins (Navarro-Soto et al., 2015).

5.2.1. *Brucella* spp. lipopolysaccharide

In smooth species of *Brucella*, lipopolysaccharide is made up of a polysaccharide that faces outward and a glycolipid component (lipid A) inserted in the outer membrane (Navarro-Soto et al., 2015). The core and the O-chain are the two components that make up a polysaccharide. *Brucella ovis* and *B. canis* (R-LPS) are O-chain-deficient by nature (OPS) (Navarro-Soto et al., 2015).

In *Brucella* S-LPS, mannose and N-formyl-perosamine link the core to the O-PS, a homopolymer of *N*-formyl-perosamine in various proportions of α1-2 and α 1-3 links (Martínez-Gómez et al., 2018). The distribution of these linkages divided strains of smooth species into A-strains and M-strains (Pérez-Sancho et al., 2015; Muñoz et al., 2022). Using monospecific anti-A and anti-M sera, biovars can be identified (Alton et al., 1988, as cited in Muñoz et al., 2022).

5.2.2. Outer membranes proteins (OMP)

Major OMPs are mostly protective antigens (Cloeckaert et al., 2002). Major OMPs in *Brucella* are Omp25 and Omp31 (both belonging to group 3), with the exception of *B. abortus*, where molecular methods have shown the missing of omp31 gene that encodes this protein (Cloeckaert et al., 2002). In addition, it has been discovered that Omp28, also known as CP28 or BP26, is an immunodominant antigen in infected cattle, sheep, goats, and humans (Gupta et al., 2010).

5.2.3. Periplasmic and cytoplasmic proteins

Proteomic analysis was used to identify immunoreactive non-LPS proteins, which were subsequently examined by ELISA using *Brucella*-positive sera and revealed no cross-reactivity with *Escherichia coli* O157:H7 nor with *Yersinia enterocolitica* (Navarro-Soto et al., 2015). Chaperonin GroES and DnaK demonstrated the highest immunological reactivity among these proteins (Navarro-Soto et al., 2015).

5.3. Virulence factors

None of the traditional bacterial virulence factors present in *Brucella* species, including exotoxins, cytolysins, a capsule, fimbriae, flagella, plasmids, lysogenic phages, endotoxic lipopolysaccharide, and inducers of host cell apoptosis (Moreno and Moriyón, 2006).

5.3.1. LPS

Contributes significantly to *Brucella* pathogenicity. In addition to interacting with lipid rafts on host cells, LPS O-polysaccharide also confers resistance to antimicrobial peptides like defensins and lactoferrin and inhibits complement-mediated bacterial lysis and host cell apoptosis (Allen et al., 1998; Jiménez de Bagüés, et al., 2004; Lapaque et al., 2005).

5.3.2. BvrR/BvrS

Is a two-component regulatory system that controls the expression of outer membrane proteins, some of which are necessary for full virulence, and modulates the host cell cytoskeleton during *Brucella* invasion (López-Goñi et al., 2002; Poester et al., 2013).

5.3.3. Cyclic β-1,2-glucans

The cyclic β-1,2-glucans are also part of the outer membrane, are necessary for *Brucella* to survive inside cells (Briones et al., 2001; Poester et al., 2013).

They generate and secrete cyclic glucans of low molecular weight. These chemicals alter the internal membranes surrounding the bacteria's lipid raft microdomain structures, preventing phagosome maturation and lysosome fusion (Arellano – Reynoso et al., 2005).

5.3.4. Type IV secretion system (T4SS)

This system is essential for intracellular survival in host cells and pathogenicity in vivo, is encoded by the elements of the virB operon (Hong et al., 2000; O'Callaghan et al., 1999). The virB operon is selectively activated within macrophages, in contrast to other type IV systems that are expressed extracellularly, and phagosome acidification is a crucial intracellular triggering signal virB expression. (Boschiroli et al., 2002). The T4SS is necessary for *Brucella* to enter its intracellular replication niche (Celli et al., 2003).

5.4. Survival of *Brucella*

Low temperatures, pH > 4, absence of direct sunshine, and high humidity are favorable circumstances for the survival of *Brucella* (Coelho et al., 2015).

In addition to surviving for 40 days in dry soil and 60 days in wet soil, *Brucella* can also survive for 144 days at 20 °C and 40 percent relative humidity, for a few months in drinking water at 4 °C to 8 °C and two and a half years at 0 °C, for 30 days in urine, 75 days in aborted fetuses, more than 200 days in uterine secretions, and for several years in frozen tissues or culture media (Coelho et al., 2015).

The organism can last 3 to 44 days in dust, 20 days on sterilized surfaces, and 30 days in tap water. The duration of resistance in wooden homes and on shelter floors is roughly four months. Survival can last up to 15 days in pastures exposed to the sun, compared to 35 days in pastures in the shade (Castrucci, 2007).

Brucellae are susceptible to most common sterilants and disinfectants (such as phenol, formol, zepherin, roccal, chlorine, etc.) (Elberg, 1958, as cited in Meyer, 1981).

Additionally, they can be eliminated by gamma irradiation, pasteurization, wet heat of 121°C (250°F) for at least 15 minutes, and dry heat of 320-338°F (160-170°C) for at least an hour. For liquids, a 10-minute boil is typically effective (Spickler, 2018).

5.5. Pathogenesis

The mouth, conjunctiva, respiratory tract, and abraded skin are entrance points (Alton and Forsyth, 1996). However, the main route of entry for *B. melitensis* in sheep and goats is through the nasopharynx (Castrucci, 2007). It then flows through the lymphatic channels to the local lymph nodes. When the immune system fails, the bacterium enters the blood, causing bacteremia and infecting the uterus. In more advanced stages, *B. melitensis* can colonize the udder causing acute mastitis (Alton, 1990; Enright et al., 1990). *B. melitensis* cells remain in blood steam for 30–45 days after infection (Castrucci, 2007).

At the cellular level, *Brucellae* alter the phagocytic cells' endosomal compartment upon entrance through the plasma membrane to enable long-term survival (Olsen et al., 2010). Moreover, *Brucellae* have the ability to survive intracellularly in either phagocytic or non-phagocytic host cells (Neta et al., 2010). They can disrupt intracellular trafficking by inhibiting the fusion of the *Brucella*-containing vacuole (BCV) with lysosome markers and guiding the vacuole towards a compartment that has rough endoplasmic reticulum (RER), which is extremely permissive to *Brucella* intracellular replication (Anderson et al., 1986; Pizarro-Cerdá et al., 1998; Pizarro-Cerdá et al., 2000, as cited in Poester et al., 2013).

Male and female genital tracts are where *B. melitensis* is most commonly found. But it can also be detected in the central nervous system, bone marrow, mammary glands, bones, renal cortex, and synovial membranes, where it causes the development of focal granulomatous lesions (Enright et al., 1990; Jubb et al., 1993).

Brucella targeting reproductive male and female organs was explained by the high concentrations of erythritol in fetal fluids, placental tissue, epididymis and semen of ruminants (Smith et al., 1962; Clark et al., 1967; Essenberg et al., 2002, as cited in Letesson et al., 2017). Indeed, *Brucellae* were discovered to use erythritol preferentially over other nutrients as a growth factor and carbon source (García-Lobo and García, 2005).

Brucella may potentially exist in a latent, non-replicative condition (Olsen et al., 2010). It is unknown what causes dormant microorganisms to "wake up". Nevertheless, some scientists have suggested that certain gestational hormones serve as a signal for the bacteria, suggesting that the moment has come to end the infectious cycle and spread through the abortion process (Moreno and Gorvel, 2005).

The chronic character of brucellosis might be explained by the inhibition of macrophage apoptosis by producing large amounts of lipopolysaccharides (LPS) during replication that form stable complexes with MHC-II proteins that interfere with peptide presentation to T cells (Moreno and Gorvel, 2005).

The mechanisms of *Brucella*-induced abortions are poorly understood, but two hypotheses have been suggested. The first one is that placentitis would prevent the delivery of nutrients to the fetus resulting in fetal stress and death (Poester et al., 2013). The second hypothesis consists in the fact that changes in the hormonal synthesis induced by *B. melitensis* in order to promote its growth could contribute to abortion (Osman et al., 2016).

5.6. Immune response

The specific host defences against *Brucellae* resemble those against other intracellular bacteria and are both humoral (antibody-mediated) and cell-mediated (Alton and Forsyth, 1996). However, *Brucella* produces a diminished innate immune response and a slower rate of dendritic cell maturation and activation when compared to other Gram-negative bacteria, which may hinder the establishment of adaptive immunological responses (Olsen and Palmer, 2014).

Nevertheless, cell-mediated response is the main element in the defense against *Brucellae* (Alton and Forsyth, 1996). It has been demonstrated that macrophages process brucellar antigen and then present it to T lymphocytes, which therefore release lymphokines (Alton and Forsyth, 1996).

Moreover, IL-12 produced by cells in the innate immune system leads to a Th1 response and induction of interferon gamma, which activates macrophages to extensive potent bactericidal activity, especially in the foci of infection that result in granuloma formation (Alton and Forsyth, 1996; De Figueiredo et al., 2015). The cytokines released by many cell types, such as colony-stimulating factors, tumor necrosis factor, and interleukin-1, increase this inflammatory response (Alton and Forsyth, 1996).

The humoral serological response has not been well studied in small ruminants, but a very close resemblance to cattle has been suggested.

Most protective antibodies in smooth-species infection are elicited by immunodominant antigen S-LPS that most serological tests are based on its antigenic properties (Cardoso et al., 2006; Nielsen and Yu, 2010; Figueiredo et al., 2015).

Generally, *Brucellae* cause an early IgM isotype antibody response, which can be delayed, but typically manifests 5 to 15 days after exposure (Beh, 1973; Allan et al., 1976).

However, the timing of IgM production depends on a number of elements: the route of exposure, the dose of bacteria and the health status of the animal (Beh, 1973, 1974; Allan et al., 1976). The IgG1 isotype of antibody is produced extremely quickly after the IgM antibody response, and then IgG2 and IgA are produced thereafter (Beh, 1974; Nielsen et al., 1984).

6. Review of brucellosis in small ruminants

6.1. Clinical signs, source of infection and transmission

Brucellosis in animals is a chronic disease that affects sexually mature animals and is characterized by reproductive issues (Megid et al., 2010).

The predominant clinical manifestations of *B. melitensis* infection in females include abortion during the final two months of gestation, placenta retention, and delivery of weak newborns that die in the first few days after birth (Megid et al., 2010). The cotyledons of the aborted placenta may be grey necrotic and edematous (Blasco et al., 1990; Aldomy et al., 1992). Animals usually only have one abortion (Megid et al., 2010).

The mammary gland is a typical location of infection in goats and sheep (Olsen and Palmer, 2014). The multinodular hardness of the afflicted mammary gland may be accompanied by watery, clotted milk (Cutler et al., 2005)

The testis, epididymis, seminal vesicle, and deferent ducts can all become infected in male goats. Reduced fertility is frequently a natural consequence of male genital infection (Olsen and Palmer, 2014).

A general reduction in herd fertility, a rise in stillbirths, a decline in milk production, and an increase in the culling of males due to chronic lesions in the reproductive organs are all signs of brucellosis within the herd (Haughey et al., 1968; European Commission, 2001).

Large amounts of *Brucella* will be released into the environment by infected females who have given birth or aborted contaminating pastures, soil, and water (FAO/OMS, 1986; Alton, 1990). Thus, the placenta, fetuses, and fetal fluids contain huge amounts of excreted bacteria, making material from an abortion the primary route of transmission (Castrucci, 2007). In goats, *Brucella* can continue to be shed through the vaginal fluid for up to two or three months after the abortion or parturition, whereas in sheep it lasts for just approximately three to four weeks (FAO/OMS, 1986; Alton, 1990). Additionally, shedding of bacteria through milk lasts longer in goats than in sheep (Olsen and Palmer, 2014).

However, in subsequent pregnancies, the infected females will continue to shed bacteria through the placenta, vaginal secretions, and milk despite having a healthy delivery (FAO/OMS, 1986; Alton, 1990).

Transmission may occur among mature animals venereally or by ingestion or inhalation of bacteria in infected material (Moreno and Gorvel, 2005). Although less frequently, *B. melitensis* can be transmitted vertically in utero or through colostrum (Grilló et al., 1997).

6.2. Risk factors

Age is considered one of the individual factors that can be linked to brucellosis (Coelho et al., 2015). Older animals were found be significantly associated to brucellosis (Abdulhameed et al., 2020; Buhari et al., 2020; Tulu et al., 2020; Ullah et al., 2020; Gompo et al., 2021). Since vulnerability rises after sexual maturity and pregnancy, brucellosis has been regarded as a disease of adult animals (Castrucci, 2007; Coelho et al., 2015). Moreover, the likelihood of contracting brucellosis was shown to be greater in female ruminants, which might be linked to the innate biology of the microbes and their affinity for the tissues of the fetus (Coelho et al., 2015).

In Latin America and Malta, where sheep are not very affected, the goat is thought to be the main host of *B. melitensis*, however in the Mediterranean regions, both sheep and goats are equally infected (FAO/WHO, 1986; Alton, 1990; Leon, 1994).

The significance of *B. melitensis* infection in sheep and goats can be influenced by regional differences that can be related to husbandry practices and susceptibility of local sheep breeds (Seria et al., 2020). Dairy sheep and goats are particularly prone to the infection (Coelho et al., 2015).

Management practices and environmental factors have a significant impact on the spread of the disease. While open-air parturition in a dry environment reduces transmission, kidding or lambing in cramped, gloomy enclosures promote the spread of the organism (Seria et al., 2020). Seria et al. (2020) state that when *B. melitensis* enters a flock or herd that has never been exposed to it or received vaccinations, the abortion rate is significant; however, it is substantially lower in flocks where this disease is enzootic.

The dogs that protect herds may potentially contract the disease, therefore, increasing the risk of the disease persistence (Alamian and Dadar, 2020).

Dogs, cats, and other wild carnivores like foxes or wolves play a significant role as mechanical disseminators because they transfer contaminated fetuses or placentas from abortions in infected herds and flocks (Coelho et al., 2015). Additionally, wild ruminants such as chamois and ibex in the Alps of France and Italy and *Camelus dromedirus* in the Middle East and llamas in South America that come into encounter with diseased sheep or goats may contract *B. melitensis,* sustaining the infection in the environment (Godfroid, 2002; Coelho et al., 2015).

Herd size is a significant risk factor for brucellosis seropositivity, being higher in large herds or flocks (Edao et al., 2020; Tulu et al., 2020; Zewdie, 2020; Gompo et al., 2021). Additionally, presence of multiple species in the farm increases greatly the transmission and persistence of the disease (Musallam et al., 2015; Tulu et al., 2020). Common grazing also was proven to be of significant importance in disseminating the brucellosis (Abdulhameed et al., 2020). Practices such as lending ram among herdsmen and introduction of animals of unknown health status were revealed to be major risk factors in transmission of brucellosis (Musallam et al., 2015; Abdulhameed et al., 2020).

However, certain practices have been shown to be effective in preventing the disease such as isolation of sick and aborted or parturient females, cleaning and disinfection of the farm, quarantine of the newly introduced animals and proper discharge of abortion materials (Blasco, 1997; Reviriego et al., 2000; Al-Talafhah et al., 2003; Islam et al., 2013; Musallam et al., 2015).

Brucellosis has been linked to blood-sucking insects in some cases (Coelho et al., 2015). Indeed, *Brucella* was isolated from eggs, larvae and adults of ticks and flies (Coelho et al., 2015; Wang et al., 2018; Jiang et al., 2019; Huang et al., 2020; Zhang et al., 2021). These insects are believed to contribute immensely in outbreaks and the persistence of brucellosis in the Inner Mongolia regions of China (Huang et al., 2020).

6.3. Diagnosis of animal brucellosis

In active state of the disease, animal brucellosis can be diagnosed by isolation and identification of the bacterium using molecular or bacteriological assays (Seria et al., 2020).

However, in chronic disease, other immunological (allergic test) and serological tests, which can be both screening and confirming tests, are used for brucellosis diagnosis (Quinn et al., 1994).

6.3.1. Direct diagnosis

Agent identification techniques are usually utilized to confirm clinical cases or suspect cases, particularly in low-prevalence or almost-free zones (OIE, 2022b).

6.3.1.1. Staining methods

Brucella is resistant to decolourisation by weak acids and thus stain red by the Stamp's modification of the Ziehl–Neelsen's method or modified Köster (Alton et al., 1988). Staining procedures indicate evidence of brucellosis. However, these methods have a low sensitivity in milk and dairy products and reduced specificity due to cross-reaction with some bacteria like *Chlamydia abortus* and *Coxiella burnetii* (Godfroid et al., 2010; OIE, 2022b). Nonetheless, results should be confirmed by culture (OIE, 2022b).

6.3.1.2. Culture

Samples from uterine discharges, aborted fetuses, udder secretions, or certain tissues, such as lymph nodes and male and female reproductive organs, should be cultured in order to isolate *Brucella* spp. (Godfroid et al., 2010; OIE, 2022b).

Some dehydrated basal media are available for the isolation of *Brucella*, among others, *Brucella* medium base, tryptose (or trypticase)–soy agar (TSA), (SDA) or glycerol–dextrose agar (OIE, 2022b). Castañeda's medium is recommended for the isolation of *Brucella* from blood and other body fluids or milk (Alton et al., 1988).

A combination of the following tests can be used to identify *Brucella* organisms to the species and biovar level: organism morphology after Gram or Stamp's staining; direct observation of colonial morphology; growth characteristics; urease and oxidase tests and slide agglutination testing with polyclonal anti-*Brucella* serum (OIE, 2022b). Extensive tests are needed to identify species and biovars, such as phage lysis and agglutination with anti-A, anti-M, or anti-R monospecific sera (OIE, 2022b).

Bacterial isolation is always required for the biotyping of strains (Godfroid et al., 2010).

6.3.1.3. PCR

Polymerase chain reaction (PCR) methods can also be used to demonstrate the agent in various biological samples (Bricker, 2002; Whatmore and Gopaul, 2011, as cited in OIE, 2022b), but the sensitivity of these approaches may be low with respect to classical bacteriology because of limitations around sample volume (OIE, 2022b). However, the PCR, including the real-time format, provides an additional means of detection and identification of *Brucella* spp (OIE, 2022b). Several molecular methods with different characteristics were developed over the years, among others, PCR restriction fragment length polymorphism (RFLP) and Southern blot, AMOS-PCR, multiplex PCR assay (Bruce-ladder), multiplex PCR assay (Suis-ladder), approaches based on single nucleotide polymorphism (SNP) (OIE, 2022b).

Those classical typing methods are able to discriminate between biovars of *Brucella*, but single-nucleotide polymorphisms (SNP), multi-locus sequence analysis (MLSA), and multiple locus variable (number of tandem repeats) analysis (MLVA) have the ability to distinguish strains within a given biovar, allowing molecular epidemiological analysis (Godfroid et al., 2010).

Several *Brucella* genes were considered as potential targets for typing scheme, among others, outer membrane protein (omp) typing such as Omp2, Omp25 and Omp31genes and IS711 typing (Cutler et al., 2005).

6.3.2. Indirect diagnosis

No single serological test is appropriate in each animal species and all epidemiological situations, and some of these tests are not adequate for diagnosing brucellosis caused by rough species (OIE, 2022b).

Therefore, consideration should be given to all factors that impact on the relevance of the test method and test results such as vaccination and cross-reaction with some bacteria like *Yersinia enterocolitica* O:9 or *Escherichia coli* O157:H7 (Navarro-Soto et al., 2015; OIE, 2022b). Therefore, the reactivity of samples that are positive in screening tests should be assessed using an established confirmatory or complementary strategy (OIE, 2022b).

Numerous immunological tests were invented, experimented and used for diagnosis of animal brucellosis. However, in this chapter, we will describe only those cited in the OIE Terrestrial Manual and applied for diagnosis of the disease in small ruminant (OIE, 2022b):

6.3.2.1. Buffered *Brucella* antigen tests (BBAT)

The Buffered Antigen Plate Agglutination test (BPAT) and Rose Bengal test (RBT) are agglutination-based techniques.

The most effective agglutinin is IgM isotype when the pH is neutral or slightly below neutral (Rice and Boyes, 1971; Corbel, 1972; Nielsen et al., 1984, as cited in Nielsen, 2002). BPAT and RBT use *B. abortus* S99 or S1119.3 cell antigen stained with Rose Bengal or Brilliant Green and Crystal Violet combined, respectively, suspended in a buffer that, when combined with the proper volume of serum, yields a final pH of 3.65 (Nielsen and Yu, 2010). Low pH inhibits some IgM agglutination and promotes IgG1 agglutination, lowering non-specific reactions as a result (Corbel, 1972, 1973; Allan et al., 1976, as cited in Nielsen, 2002).

6.3.2.2. Complement fixation test (CFT)

CFT detects anti-*Brucella* antibodies that can activate complement (Godfroid et al., 2010). CFT measures IgM and IgG and is used as a confirmatory test because it is more specific but less sensitive than RBT and ELISA (Bosilkovski ,2015; OIE, 2022b). However, CFT is technically difficult and requires numerous reagents (Nielsen and Yu, 2010).

6.3.2.3. Enzyme-linked immunosorbent assays (ELISA)

Most indirect ELISAs use S-LPS antigen (Nielsen and Yu, 2010). The majority of iELISAs detects mainly IgGs or IgG sub-classes (Godfroid et al., 2010). They are very sensitive but have the same limitation of cross reaction with other bacteria (Godfroid et al., 2010; OIE, 2022b). ELISA is used on sera and on pooled milk samples. This technique has replaced the Milk Ring Test due to better performance and use (Nicoletti, 2010).

Whereas, competitive ELISA is slightly less sensitive than iELISA but more specific due the use of monoclonal antibodies directed against specific epitopes of the *Brucella* LPS (Godfroid et al., 2010; Nielsen and Yu, 2010). Therefore, cELISA is used to differentiate vaccinal antibodies from antibodies post- infection (Nielsen and Yu 2010).

6.3.2.4. Fluorescence polarization assay (FPA)

The FPA is highly precise and quick, and the sensitivity/specificity can be adjusted by changing the cut-off value between positive and negative reactions to offer both a highly sensitive screening test and a highly specific confirmation test (Minas et al., 2007; Nielsen and Yu, 2010). The FPA can lessen but not completely eliminate responses brought on by residual antibodies created in response to vaccination (Nielsen et al., 1996). Moreover, Specificity of FPA regarding false positive reactions is undetermined in cattle and small ruminants (OIE, 2022b). The OIE considers this test as a legitimate procedure for importing and exporting pigs, small ruminants, and cattle (OIE, 2022b).

BBATs (Rose Bengal Test [RBT] and the buffered plate agglutination test [BPAT]), ELISA, and FPA are considered to be appropriate screening tests for the control of brucellosis at the national or local level (OIE, 2022b).

6.3.2.5. Native hapten and cytosol protein-based tests (ruminants only)

Native hapten and cytosol proteins used as antigen to increase specificity particularly in vaccination contexts in cattle and small ruminant (Nielsen and Yu, 2010; OIE, 2022b). The said antigens used mostly in precipitation tests that are divided into two formats: Agar gel immunodiffusion (AGID) and radial immunodiffusion (RID) (Nielsen, 2002; Nielsen and Yu, 2010; OIE, 2022b). False positive reactions in cattle are eliminated using these tests (Muñoz et al., 2005).

6.3.2.6. Brucellin skin test

The brucellin skin test, based upon intradermal inoculation of LPS free antigen preparations is more specific than conventional serological assays by eliminating cross-reaction in ruminants, camels and swine (Pouillot et al., 1997; Saegerman et al., 1999; Bercovich, 2000, as cited in Cutler et al., 2005; Godfroid et al., 2010; OIE, 2022b). However, it has the same drawback of diagnosis interference in vaccination contexts (OIE, 2022b).

This test by itself is not suitable for use in international trade or as a standalone diagnostic test. However, it can be advised for herd/flock surveillance in brucellosis-free areas (OIE, 2022b).

6.4. Treatment

Despite antibiotics' capacity to lessen clinical indications, control by treatment is typically unsuccessful due to the organisms' intracellular sequestration in the lymph nodes, mammary glands, and reproductive organs (Bayu, 2018; Spickler, 2018). Due to this reason as well as the zoonotic hazards, treatment of diseased animals is discouraged (Spickler, 2018).

6.5. Control and eradication of small ruminant brucellosis

It is impossible to prevent human brucellosis without controlling the animal disease. Thus, control, eradication and prevention of brucellosis is a "One Health" strategy that should be addressed by public and animal health authorities (Pérez-Sancho et al., 2015).

Before implementation of a control/eradication program, several factors should be considered: flocks should be under strict surveillance and movement control, animals individually identified and an efficient and well-organized veterinary service for surveillance and laboratory testing in place (Alton, 1990; Nicoletti, 1993, as cited in Minas, 2006; Blasco, 2010). Other factors impact greatly the outcome of the control program, among others, involvement of all stakeholders, the infection rate in the herds, type of husbandry and economic resources (Benkirane, 2006; European Commission, 2001; Pérez-Sancho et al., 2015).

6.5.1. Control strategies

Combining three main approaches has been shown to be an efficient way to control brucellosis in domestic animals (Nicoletti, 2010; Pérez-Sancho et al., 2015): **1.** Strict biosecurity at the farm level **2.** Test-and-slaughter policy **3.** Immunization of the susceptible population.

6.5.1.1. Strict biosecurity at the farm level

According to Benkirane (2006), Minas (2006) and Muñoz et al. (2022), general management practices and hygienic measures have to be applied simultaneously for the control program to be effective:

- Isolation of animals in the third trimester of pregnancy.

-Disinfection of infected premises and materials.

-Destruction of abortion products as well as removal of aborted females contributes to lower the contamination.

-Implementation of quarantine before the introduction of new animals.

-Separation of animals with an unknown/uncertain status.

-Strict quality/sanitary control of semen.

6.5.1.2. Test-and-slaughter policy

This policy is defined by testing animals and eliminating positive reactors by slaughter. Before beginning this approach to eradicate brucellosis, it is important to make sure that the epidemiological situation is favourable, the required facilities and financial resources are available, there is a pool of healthy replacement animals and the resources and ability for on-going surveillance would be available for a significant amount of time in addition to the complete cooperation of the farmers (Minas, 2006).

6.5.1.3. Immunization of the susceptible population

The appropriate application of vaccination will in any case result in a suppression of the infection pressure and has been shown to reduce the zoonotic spread of the disease (European Commission, 2001). Only a high-quality vaccination that has been given to at least 80% of the animals at risk will provide adequate protection (Garrido, 1992, as cited in Minas, 2006). The most effective vaccine currently available for the prevention of brucellosis in sheep and goats is the live, attenuated *B. melitensis* REV-1strain (Minas, 2006; OIE, 2022b). However, the REV-1 has two main drawbacks: the abortifacient effect and the long-lasting serological response that causes diagnostic interference (Minas, 2006; Pérez-Sancho et al., 2015).

6.5.2. Control scheme

Control of brucellosis refers to any efforts made to bring the disease's incidence and prevalence in a given animal population down to a manageable level, minimizing its effects on public health and the local economy (Minas, 2006). Most authors (Benkirane, 2006; Minas, 2006; Blasco, 2010) agreed on the following control/eradication scheme of *B. melitensis* infection in small ruminant based on the prevalence rate:

1. If the prevalence of brucellosis is high (>5–10%) vaccination of all animals (mass vaccination) is highly recommended.

2. If the prevalence is between 1 and 5%, combination of vaccination of young animals and test-and-slaughter approach should be applied.

3. If the prevalence is less than 1%, test-and-slaughter policy should be implemented to eradicate the disease.

Chapter 2

Modeling of Infectious Diseases in Epidemiology

1. Definition

Mathematical models of infectious diseases are defined by mechanistic description of the transmission of infection between two individuals or category of population differentiated by their status of the disease (Susceptible, infectious, removed/immune) (Kretzschmar and Wallinga, 2009) (Figure 3). The transmission process over time between compartments or individuals is described by mathematical equations.

According to Earn (2008):

- Susceptible: individuals who are not immune to the disease and might become infected if exposed to the infectious agent.
- Infectious: infected individuals who can transmit the disease to susceptible individuals after an effective contact.
- Removed: individuals who are immune to the infection, and consequently do not affect the transmission dynamics in any way when they contact other individuals

2. History of modeling

The first model was developed by "Daniel Bernoulli" in 1766 describing mathematically the effect of smallpox variolation on life expectancy (Dietz and Heesterbeek, 2000). Later, in 1906, Hamer was the first to realize that the decrease in the susceptible population could cease the epidemic. Meanwhile, Sir Ronald Ross studied the effectiveness of multiple intervention measures for malaria via the use of mathematical modeling (Kretzschmar and Wallinga, 2009). Afterward, Kermack–McKendrick in 1927, Lowell Reed and Wade Hampton Frost in the 1920s have pioneered the epidemic models using compartmental models known as Kermack–McKendrick and Reed-Frost models (Abbey, 1952; Brauer and Castillo-Chávez, 2012). Related researches have continued since then. However, until the end of the twentieth century that mathematical modeling became a known tool used in designing strategies in public health issues (Kretzschmar and Wallinga, 2009).

3. Advantages of modeling

Mathematical modeling in epidemiology provides a profitable mean to study diseases in case of impracticability of field investigation or experimentation (Thrusfield, 2007).

For instance, it permits simulation of experiments entitled unethical in human beings (Costa et al., 2021).

Moreover, modeling allows the identification and prediction of patterns in epidemics as well as simulating the impact of policies and intervention such as medical treatment, vaccination, quarantine, social distance and hygiene and management measures (Thrusfield, 2007; Costa et al., 2021). Implementation of control and intervention strategies in real life could have cost billions without warranted success. In addition, the simulation grants the capacity of previewing different alternatives before choosing the optimum policy.

4. Models' classification

4.1. Density and prevalence models

This classification was created based on the type of the infectious agent, which is divided into two categories: micro-parasites (e.g., viruses and bacteria) and macro-parasites (e.g., helminths and arthropods) (Thrusfield, 2007).

Density models regard the absolute number of the infectious agent within the host or in the environment which the case of macro-parasites due to the fact that they can be enumerated (Thrusfield, 2007). However, prevalence models regard the infection status in different host groups (e.g., susceptible and immune) which are frequently used to study micro-parasites (Thrusfield, 2007).

4.2. Deterministic and stochastic models

Deterministic models do not take into consideration variability and uncertainty of the parameters. They use fixed input parameters and the results are in fixed outputs. Differential equations are used to represent such models. Unlike stochastic models that use probabilistic equations which consider the uncertainty of the studied parameters and probability of the dynamic of the disease resulting in a distribution of outcomes with confidence intervals (Thrusfield, 2007; Marion and Lawson, 2008; Costa et al., 2021).

4.3. Agent based and meta-population models

Agent or individual based models consider the detailed movement of each individual in the population underpinning the need for massive input data making it onerous to work at large scale (Ajelli et al., 2010). However, meta-population models consider the movement and the dynamic of the disease between groups, patches or subpopulation over time and space providing wide-reaching framework (Van den Driessche, 2008; Ajelli et al., 2010; Chen et al., 2019).

4.4. Continuous and discreet time models

The mathematical models can be developed at a discrete time or a continuous time depending on the evolution of the system over time at a discrete or at a continuous pace (Wacker and Schlüter, 2020).

5. Disease transmission models

At the beginning of a disease outbreak, the number of the infected (infectious) is small where the transmission of infection is a stochastic event that would suitably be described by a stochastic-branching process, allowing therefore to distinguish between a minor outbreak and a major outbreak regardless of the value of the basic reproduction number. When the epidemic evolves, the size of subgroups of the population will be large enough that the mixing of individuals would be homogeneous where the compartmental epidemic models would be suitable to describe this phase of the epidemic (Brauer and Castillo-Chávez, 2012).

5.1. Epidemic models

5.1.1. Definition of an epidemic

An epidemic is determined by a sudden outbreak of a disease in a short period of time affecting a considerable fraction of the population to disappear afterwards (Brauer and Castillo-Chávez, 2012). During the evolution of the epidemic, the susceptible population drops due to the removal of infected individuals by death or due to the rise of the immunity post-infection, causing therefore the cease of the epidemic (Thrusfield, 2007).

Epidemics evolve on a brief period that effect of some demographic's events such as death, birth and immigration may be neglected (Brauer and Castillo-Chávez, 2012).

5.1.2. Basic reproduction number

The basic reproduction number R_0 is defined as the expected average number of secondary cases generated by a typical infective individual in a completely susceptible population during its entire infectious period (Diekmann et al., 1990; Van den Driessche, 2017).

In epidemic evolution, the basic reproduction number R_0 ascertains a threshold, if $R_0 > 1$, a typical infective transmits the infection on average to more than one susceptible individual which causes an epidemic, and if $R_0 < 1$, a typical infective infects on average less than one susceptible individual, therefore, the infection will die out (Cintrón-Arias et al., 2009). Mathematically, *R*0 is a threshold for stability of a disease-free equilibrium (DFE) and linked to the peak and total size of an epidemic (Van den Driessche and Watmough, 2008).

5.1.2.1. Computation of R0

5.1.2.1. 1. Anderson and May method

The basic reproduction number is usually determined by three factors: the contact rate in the population, transmission probability during the contact and the duration of infectiousness (Thrusfield, 2007; Delamater et al., 2019).

According to Jones (2007), the R_0 is a dimensionless number that can be calculated as follows in simple compartmental models without background death rate:

R_0 = (Contact rate) X (Transmission probability during the contact) X (Duration of infectiousness).

5.1.2.1.2. Next generation matrix

This method is used in structural complex models with more than one compartment of infected individuals (Van den Driessche, 2017).

The definition is given according to Van den Driessche (2017) based on Diekmann et al. (1990), and Van den Driessche and Watmough (2002):

- The whole population is divided into n compartments including m<n of infected compartments.
- Let $X = (X_1, X_2, \ldots\ldots X_n)^T$ be the number of individuals in each compartment at time T.
- Assume that the DFE (X_0) exists and is stable in the absence of disease.
- Assume that the linearized equations for X_1; …; X_m at the DFE (disease free-equilibrium) decouple from the other equations. These equations written as follows: $\frac{dxi}{dt} = Fi(x) - Vi(x)$ For i=1; 2; …; m.
- Fi(x) is the rates of appearance of new infections in compartment i, and Vi(x) is the rates of other transitions between compartment i and other infected compartments.

For DFE X_0, $F = \frac{\partial Fi(X0)}{\partial Xj}$ and $V = \frac{\partial Vi(X0)}{\partial Xj}$ For i≤1, j≤ m. FV^{-1} is the next generation matrix for the system at the disease-free equilibrium.

The basic reproduction number is defined as the spectral radius of the next generation matrix FV^{-1} (Diekmann et al., 1990). According to the same authors, the spectral radius of a matrix FV^{-1}, denoted ρ (FV^{-1}), is the maximum of the moduli of the eigenvalues of FV^{-1}, giving therefore:
R0= *p* (FV^{-1}).

5.1.3. Simple Kermack–McKendrick epidemic model

The Kermack–McKendrick model is a compartmental model based on assumptions on the rates of circulation between different classes of subgroups related to the disease status (Brauer and Castillo-Chávez, 2012). Also, it is known as the SIR model that describes the dynamic of a disease that confers immunity against reinfection indicating the movement of individuals from the susceptible compartment S to the infective compartment I to the removed class R (Figure 3) (Brauer, 2008; Brauer and Castillo-Chávez, 2012).

The independent variable in this model is the time t, and the rate of movement from class to another is determined by the derivatives of the size of each compartment with respect to time, resulting in the model being represented by the differential equations (Brauer and Castillo-Chávez, 2012).

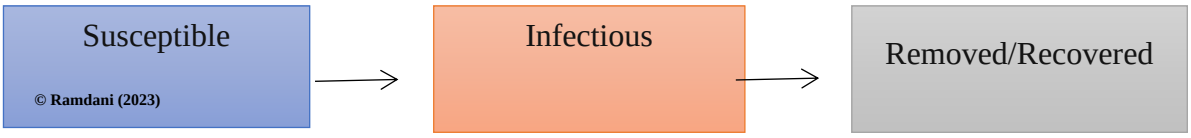

Figure 3: Flow chart of the dynamic of transmission of an infectious disease in SIR model

Differential equations of the SIR model:

$S' = -\beta SI$;

$I' = \beta SI - \alpha I$;

$R' = \alpha I$

α is the rate of departure from the infective class through recovery and β denotes the transmission rate.

The flow chart (figure 3) and the equations represent the SIR model that is based on the following assumptions (Brauer and Castillo-Chávez, 2012):

1. A typical member of the population (N) makes an effective contact to transmit the disease to *BN* individuals per unit of time (mass action).
2. An infected individual recovers and leaves the infected compartment at the rate αI per unit of time.
3. The total population is fixed and constant N and there is no disease deaths.

5.1.4. Branching-process disease-outbreak model

Stochastic-branching process describes the beginning of the epidemic where the distribution of contacts is heterogeneous due to the small number of the infectives (Brauer, 2009).

The branching process models express a different behavior compared to the Kermack–McKendrick model.

Consequently, if $R0 < 1$, the probability that the infection spread will cease is 1. However, if $R0 > 1$, there is a probability that the spread of infection will rise at the beginning but will only cause a minor outbreak and will cease before generating a major epidemic (Brauer and Castillo-Chávez, 2012).

5.1.5. Network models

Compartmental models cannot determine the contact between individuals, which is considered a crucial aspect in the dynamic of spread of some diseases, among others, sexually transmitted diseases (Kretzschmar and Wallinga, 2009). Including the duration of partnerships led to the development of network models shaped by graphs in which nodes represent individuals and the links (lines) represent their contacts (Keeling and Eames 2005, as cited in Kretzschmar and Wallinga, 2009). Network modeling was also used to study the dynamic of transmission of respiratory diseases (Meyers et al., 2003, as cited in Kretzschmar and Wallinga, 2009).

5.1.6. SEIR model

The SEIR model is a derivative from the Kermack–McKendrick model. The latent period where the exposed individual contracts infection but cannot transmit the infection, yet is added to the mathematical model forming a fourth compartment denoted E (Aron and Schwartz, 1984). As a result, the exposed individuals would leave the compartment to be infectious at the rate kE (k^{-1}: the mean duration of the latent period).

The SEIR model is described as follows:

$S' = -\beta SI$;

$E' = \beta SI - kE$;

$I' = kE - \alpha I$;

$R' = \alpha I$

5.1.7. Models with disease deaths

Epidemic of disease that causes mortality but it confers immunity against reinfection. Thus, the population *N* would be a decreasing function (Brauer and Castillo-Chávez, 2012). Furthermore, the removed individuals from the infectives class (αI) shall be divided into those who die from the disease and those who recover and become immune against reinfection (Brauer and Castillo-Chávez, 2012). The fraction *f* is assumed to represent the members recovering from the disease and the fraction (1-*f*) represent the members that die from the disease (Brauer and Castillo-Chávez, 2012).

S, I and N are the variables of the three-dimensional model, with N=S+I+R

$S = - \beta(N)SI,$

$I = \beta(N)SI - \alpha I,$

$N = - (1 - f) \alpha I.$

5.1.8. Vaccination model

An example was given by Brauer and Castillo-Chávez (2012) related to the vaccination against seasonal influenza before the occurrence of the outbreak. Accordingly, data of vaccination was added to the SIR model. N is denoted as the total size of the population. ɣ is the fraction of vaccinated individuals before the outbreak of the disease. Consequently, two subpopulations would be defined: a subpopulation of vaccinated members of size $Nv = \gamma N$ and a subpopulation of unvaccinated members of size $Nu = (1-\gamma) N$.

Vaccinated individuals are assumed to have reduced susceptibility to infection by a factor σ, $0 \leq \sigma \leq 1$, with $\sigma = 0$ indicating a perfect effectiveness of the vaccine and $\sigma = 1$ indicating that the vaccine has 0% effectiveness. Additionally, vaccinated members are assumed to have reduced infectivity by a factor δ. Moreover, vaccinated individuals have a recovery rate denoted αV, while unvaccinated member have a recovery rate αU.

The vaccination model is described as follows:

$S_U = -\beta S_U (I_U + \delta I_V),$

$S_V = -\sigma\beta S_V (I_U + \delta I_V),$

$I_U = \beta S_U (I_U + \delta I_V) - \alpha_U I_U,$

$I_V = \sigma\beta S_V (I_U + \delta I_V) - \alpha_V I_V.$

S_U, S_V, I_U, I_V designate the unvaccinated susceptible, the vaccinated susceptible, the unvaccinated infective, and the vaccinated infective respectively.

5.2. Endemic models

5.2.1. Effective reproduction number

The initial conditions that favor the spread of the disease change over the course of the epidemic, therefore, R_0 might not be a good measure of the transmission dynamic of the disease anymore (Van den Driessche and Watmough, 2008). The basic reproduction number would be replaced by the effective reproduction number *R* (t) which is defined as the average number of infectives contracted the infection from a single typical infective at time t introduced to a population that partially immune to the disease (Farrington and Whitaker, 2003). This parameter is used to evaluate the effectiveness of the vaccination program (Farrington and Whitaker, 2003).

$R(t) = \frac{S(t)}{N} R_0$ and $R(t) \leq R_0$ (Cintrón-Arias et al., 2009).

According to Miller (2003), endemic diseases have an average value of effective reproduction number *Re*=1, outbreaks of diseases occur when *Re* exceeds 1 and it dies out when *Re* diminishes bellow 1. Indeed, the effective reproductive number is a key epidemic parameter used to assess whether an epidemic is growing, shrinking or holding steady (Gostic, et al., 2020).

5.2.2. Model for diseases with no immunity

Other types of compartmental models that differ from SIR model based on the immune response against infectious agents. For instance, SIS model represents a disease that confers no immunity implying that the infective becomes susceptible again after recovery (Brauer and Castillo-Chávez, 2012). Diseases caused by bacterial and helminthic agents are mostly represented by such models (Brauer and Castillo-Chávez, 2012). SIS model is represented mathematically as follows:

$$S' = -\beta SI + \gamma I$$

$$I' = \beta SI - \gamma I$$

In this model, infectives move to susceptible compartment after recovery at the rate ɣI. ɣ denotes the recovery rate. β denotes the transmission rate.

5.2.3. SIR model with births and deaths

Endemic diseases must be modeled on a long-time scale; thus, births and deaths should be included (Brauer and Castillo-Chávez, 2012). Births proportional to the total population size would be included in the susceptible subgroup, whereas deaths would be proportional to each compartment size (Brauer and Castillo-Chávez, 2012). If the death and birth rates are unequal, the size of the population would grow or die exponentially (Brauer and Castillo-Chávez, 2012).

When the population (K) is closed meaning the death and birth rates (μ) are equal, the model would be formulated as follows (Brauer and Castillo-Chávez, 2012):

$S' = - \beta SI + \mu (K - S)$,

$R' = \gamma I - \mu R$.

In case of fatal endemic diseases, the R class would contain only the recovered individuals (Brauer and Castillo-Chávez, 2012). As a result, the total population size would not be constant due to the removal of dead members due to the disease; therefore, it would vary in time (Brauer and Castillo-Chávez, 2012).

The mathematical model will be represented by the following differential equations:

$S' = \Lambda - ßSI - \mu S$,

$I' = ßSI - \mu I - \alpha I$,

$N' = \Lambda - (1 - f)\alpha I - \mu N$,where:

- $N = S + I + R$, with a mass action contact rate
- A constant number of births Λ per unit time,
- A proportional natural death rate μ in each class,
- A removal rate α (including disease deaths and recovered infectives).
- f : fraction of recovered members with immunity against reinfection.

5.2.4. Herd immunity

The disease might be eliminated by the phenomenon of herd immunity that is defined by vaccination or immunization of a fraction of susceptibles to be reduced below a critical threshold, so that the spread of the infection cannot cause a large epidemic (Farrington and Whitaker, 2003).

Moreover, the basic reproduction number R0 shall be below 1 for preventing the disease from becoming endemic (Brauer et al., 2019).

If a fraction p of new born Λ(N) or another category of susceptibles is immunized effectively, the N would be replaced by N(1-p), therefore the basic reproduction number would be expressed by R0 (1-p) (Brauer et al., 2019).

For R0 (1-p) < 1, gives $1 - p < \frac{1}{R0}$ which implies that the fraction of immunized members should be $P > 1 - \frac{1}{R0}$ (Kretzschmar and Wallinga, 2009; Brauer et al., 2019).

The smallpox was successfully eradicated in the 1970s through the concept of herd immunity by vaccinating about 80% of the population given the fact that the basic reproduction number was around 5 (Kretzschmar and Wallinga, 2009; Brauer et al., 2019).

6. Equilibrium states

Equilibrium points are time-independent solutions of the nonlinear differential equation model with constant coefficients (Martcheva, 2015). According to the same author, the derivative of these points with respect to time is zero giving the equation:

$$\frac{dI}{dt} = f(I) = 0$$

The two equilibrium points are the solutions of the equation $f(I) = 0$ that consisted of $I_1 = 0$ and $I_2 = K$ (Martcheva, 2015). The equilibrium I_1 which corresponds to S = S* always exists and is referred to as a **disease-free equilibrium** meaning the disease is not present in the population (Brauer and Castillo-Chávez, 2012; Martcheva, 2015). The equilibrium I_2 exists only if $R0 > 1$ and called an **endemic equilibrium**.

R0 is a threshold parameter for the model (Van den Driessche and Watmough, 2002). If R0 <1, the DFE is locally asymptotically stable and the invasion of the population by the disease is impossible, but if R0 > 1, the DFE is unstable and invasion is always possible (Van den Driessche and Watmough, 2002).

Experimental part

Chapter 1

Cross Sectional Study of Brucellosis in Small Ruminant in El Oued District

1. Problem statement and specific objectives

Epidemiological and bacteriological data are vital in control and eradication strategies of the brucellosis. Several studies related to brucellosis mostly in man and cattle in different parts of Algeria were conducted (Aggad and Boukraa, 2006; Bachir Pacha et al., 2009; Lounes et al., 2014; Abdelhadi et al., 2015; Derdour et al., 2017; Kardjadj, 2018; Rabehi et al., 2018; Kaaboub et al., 2019; Khames et al., 2020; Yahia et al., 2020; Lounes et al., 2021).

Despite the fact that sheep and goats are common hosts of *B. melitensis*; the most pathogenic species for humans, limited epidemiological data of small ruminant brucellosis are available in Algeria (Nehari et al., 2014; Gabli et al., 2015; Kardjadj et al., 2016). Moreover, serological surveys on brucellosis in small ruminants and the associated risk factors have never been carried out in Algeria with an appropriate sampling strategy, taking into consideration the interference of vaccination on serologic testing and the imperfection of the later.

In this context, a cross-sectional study of brucellosis in small ruminant was conducted in El Oued province to better understand the epidemiology of brucellosis in this area. The main objectives of the present study were threefold:

1. To provide unbiased estimate of the prevalence of brucellosis in small ruminant flocks.
2. To identify risk factors associated to brucellosis at flock level.
3. To draw up a geographic map of brucellosis distribution in small ruminants in El Oued area.

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2. Materials and methods

2.1. Study area

El Oued is a Saharan province located in the Northeast of Algeria (Figure 4); it covers an area of 34753 km² and administratively divided into 22 municipalities (Figure 2). It is located at an altitude of 88 meters above sea level, between 33° 21' North latitude and 6° 51' East longitude. The southern part of the province is covered by sand dunes, whereas, the northern part is characterized by sandy desert with scarce vegetation and Salt Lake at the west (Chott) (DPBM El Oued, 2021). El Oued area has a very dry climate. According to the data recorded in Guemar weather station, this region is characterized by a mean annual rainfall of 70 mm and a mean annual temperature of 28.4°C (Bouselsal and Saibi, 2022).

The mean annual evapotranspiration is around 1164 mm, with a pic of 216 mm in July and a low of 216 mm in January (9.4 mm) (Bouselsal and Saibi, 2022).

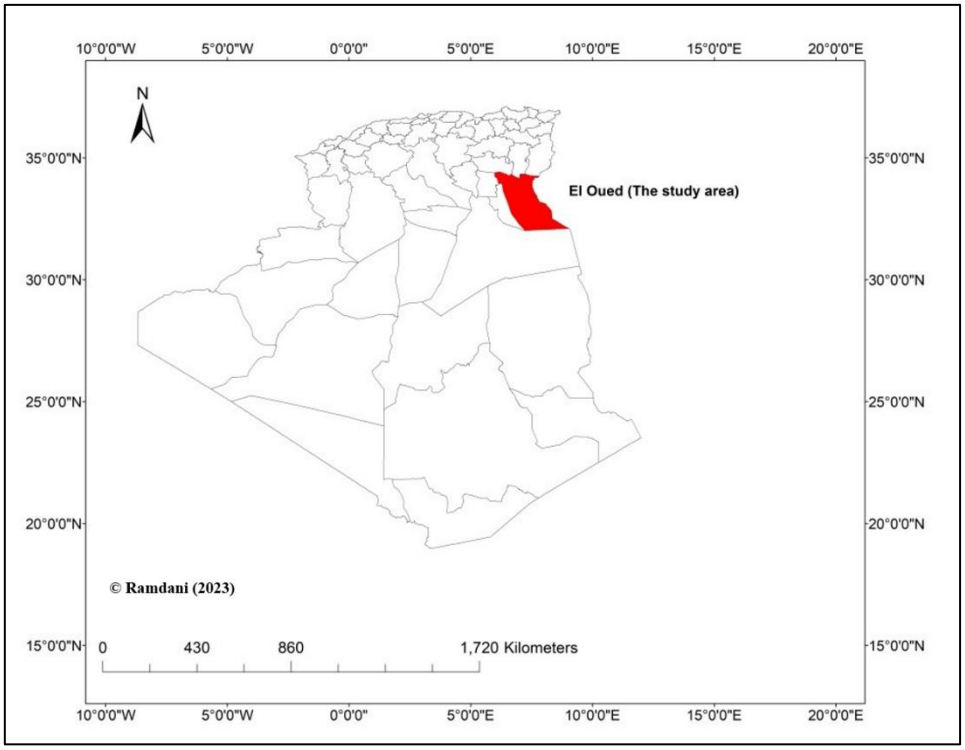

Figure 4: Map representing the localization of El Oued in Algeria

2.2. Animal populations

The total number of livestock was estimated at 1,055,027 with 93% of small ruminants. (DSA El Oued, 2020).

Four types of small ruminant farming systems are distinguished in El Oued province: 1). Smallholder dairy farming, which is an intensive husbandry system, whose predominant type of production is dairy production for household consumption, and relies mostly on family labour. 2). The second category is the extensive husbandry system which is located in the Sahara and consists of semi-nomadic system. In this system, pastoral nomads are on the move in search of grazing, water or favorable weather conditions. However, the transhumance is a seasonal practice, carried out during summer towards the steppe zones (khenchla and Oum Bouaghi). Besides, this pastoral practice is no longer free and only well-situated financially animal owners can rent meadows during estival period.

Moreover, nomads or herders do not only manage their animals, they are also paid by owners who occupy different functions to keep and take care of their animals. 3). The mixed livestock farming system (family-Saharan) which includes two sub-classes of farming system: 3.1. The first, small-flock farms for household use, except that these animals are of Saharan origin. Nomads return to their domicile during children schooling period, only one family member usually the father continues adopting the semi-nomadic lifestyle. 3.2. The second one is the breeder-fattener farming system, which consists in rearing feeble young animals born in the Sahara that could not withstand the harsh conditions of the desert; females return to the herd at reproductive age (6-8 months); however, males are fattened for slaughter. 4). Intensive fattening farming which is exclusively commercial and whose origin of animals to be fattened is heterogeneous. The organizational chart in figure 5 illustrates the different types of the farming system of sheep and goats in the study area.

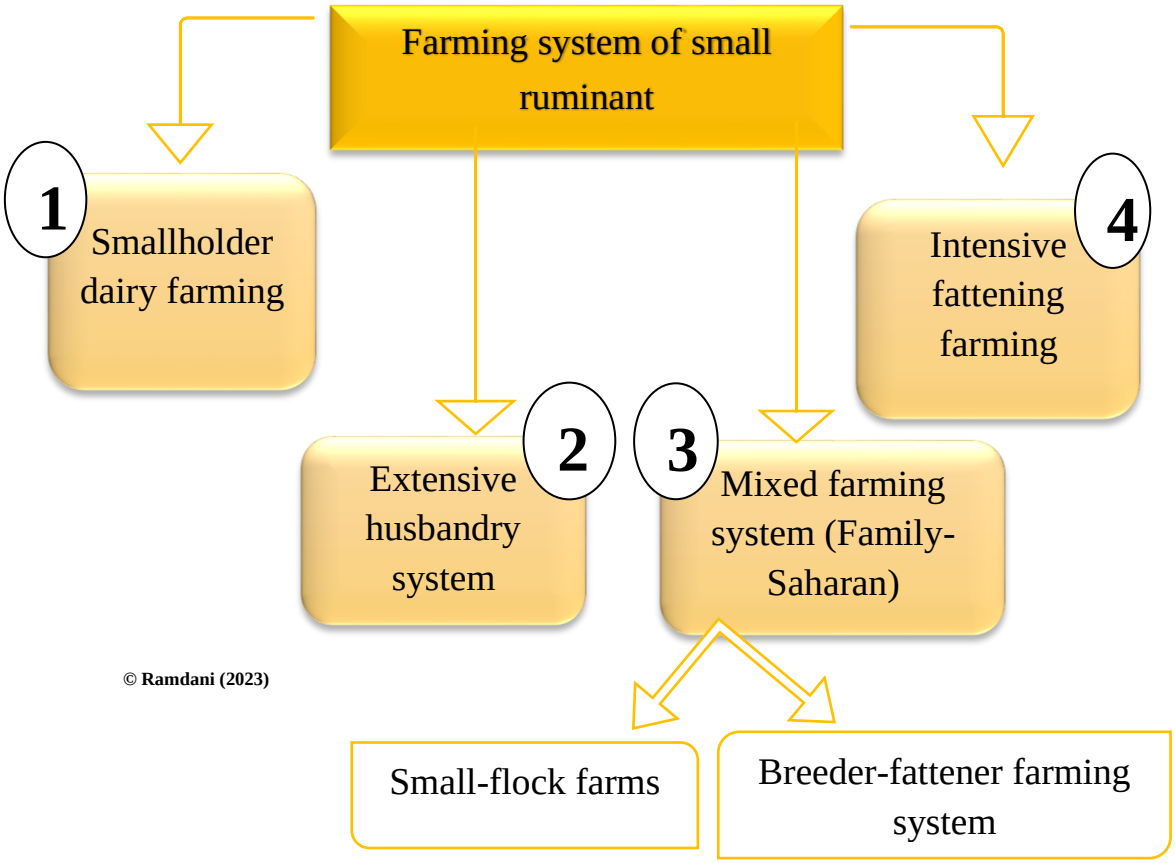

Figure 5: Organigram of framing system of small ruminant in El Oued province

2.3. Sampling strategy and sample size

In the province of El Oued, a cross-sectional study was conducted utilizing a simple random sampling approach among a few chosen herds between February 2019 and February 2021. The herd of small ruminants from all different types of production systems in the El Oued region was the primary sampling unit. Animals were the secondary sample component (sheep or goats).

Due to the limitations of the serological tests used for brucellosis screening and diagnosis, the formula below described by Humphry et al. (2004) was used to calculate the sample size in order to estimate the true herd-level prevalence of brucellosis:

$$\mathbf{HN} = \left(\frac{\mathbf{Z(C)}}{\mathbf{L}}\right)^{\mathbf{2}} \times \frac{[(\mathbf{HSENS(HTP)} + [\mathbf{1} - \mathbf{HSPEC}][\mathbf{1} - \mathbf{HTP}]) \times (\mathbf{1} - \mathbf{HSENS(HTP)} - [\mathbf{1} - \mathbf{HSPEC}][\mathbf{1} - \mathbf{HTP}])]}{(\mathbf{HSENS} + \mathbf{HSPEC} - \mathbf{1})^{\mathbf{2}}}$$

Therefore, in order to estimate a true prevalence with an imperfect test, the number of herds to be sampled (HN) was calculated using the online Epitools Calculator (Sergeant, 2018) (Figure 6):

The assumed true herd prevalence (HTP) was 3.33% (Kardjadj et al., 2016).

The herd sensitivity (HSENS) and the herd specificity (HSPEC) each chosen to be 95%.

The confidence limits (C) were chosen to be 90%, therefore the Z-score was Z =1.645.

The absolute precision L was 7%.

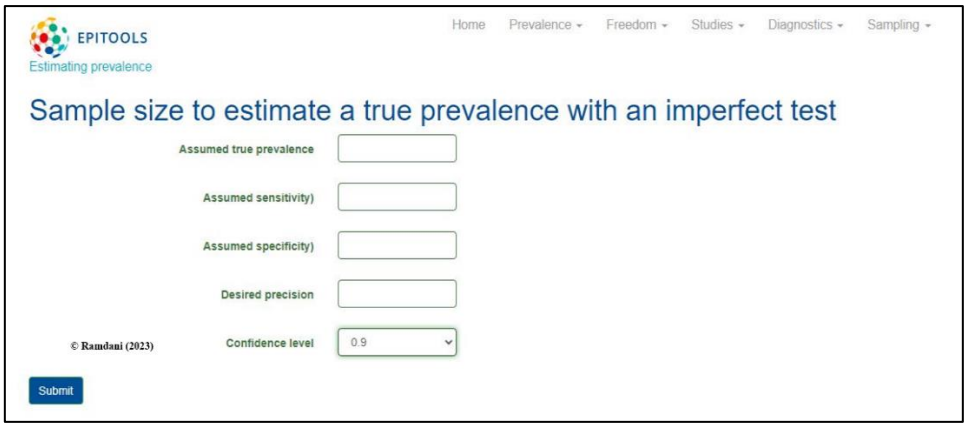

Figure 6: Calculation of number of herds to be sampled in Epitools website

Then, using the FreeCalc application in the online Epitools Calculator (Sergeant, 2018), the number of animals to be sampled per herd to achieve the HSENS and HSPEC chosen in stage one for freedom testing was calculated in accordance with the procedures described by Cameron and Baldock (1998) and Cameron (1999), using an approximation to the hypergeometric distribution (Figure 7).

The parameter inputs used were:

- The herd size =120.
- Test sensitivity (se) = 99% for Rose Bengal (RBT) and indirect ELISA (iELISA) tests used in parallel testing (Minas et al., 2007).
- Test specificity (sp) =99% for Complement fixation test (CFT) used for confirmation
- The minimum within herd prevalence= 35% (Musallam et al., 2015).
- The maximum acceptable error Type I=0.05(1− herd sensitivity) and error Type II =0.05) (1− herd specificity).

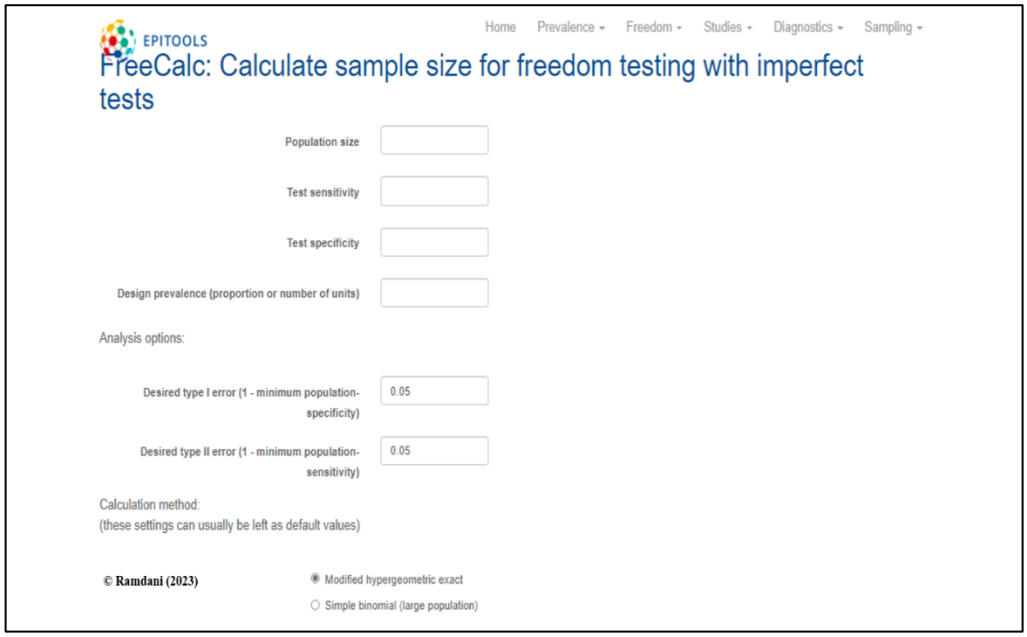

Figure 7: Calculation of number of animals per herd to be sampled in Epitools website

The main outputs were:

- The sample size =12.
- The cut-off number of positives in each herd= 01.
- Herd sensitivity (HSENS) = 96.62%.
- Herd specificity (HSPEC) = 99.38%.

From 22 municipalities, the number of herds to be sampled was divided proportionally to the number of small ruminants in each municipality, generating 51 herds after rounding. Number of sheep and goats per municipality was provided by the agricultural services of El Oued (DSA El Oued, 2018) (Appendix 1). Animals and herds from which to draw were chosen at random. The only animals sampled were those older than six months. Verbal agreement was offered by the owners of livestock in regard of the administration of the questionnaire (Appendix 2) and the collection of biological samples.

2.4. Sera and data collection

From fifty-one small ruminant flocks (n=51), six hundred and twelve (612) blood samples were collected from sheep and goats (sheep=280, goats=332) by jugular venipuncture in 5 ml labeled vacutainer tubes. Sera were recuperated in labeled Eppendorf tubes after centrifugation at 3000 rpm for 5 min, and stored at -20°C until being tested (Figures 8 and 9).

Figure 8: Blood drawing by jugular venipuncture

Figure 9: Collecting and labeling sera in Eppendorf tubes with individual animal identification number

A standardized and tested questionnaire with mainly closed-ended questions was administered to gather information of factors that might be associated to brucellosis in flocks. Questions about the socioeconomic situation of the owner such as the employment time, the location, characteristics and composition of the flock (animal species in the farm, production type, production system, the herd size and composition in term of origin of animals), the herd's history of health issues related to brucellosis like abortions, retained placenta and stillbirth, and management practices such as contact with wild animals and other herds, abortion management, isolation of unhealthy animals and parturient females, methods of herd renewing, quarantine of newly introduced animals, the frequency of cleaning of premises, visitors and the origin of the male reproducer (Appendix 2).

2.5. Serological tests

All sera were subjected to two serological tests: Rose Bengal (RBT) and indirect enzyme-linked immune-sorbent assay (iELISA). Positive sera to both tests were tested by the complement fixation test (CFT). The final result was based on CFT results.

These serological tests (RBT, iELISA and CFT) were performed at the Regional Veterinary Laboratory of El Oued province and the Management of Animal Health and Productions Laboratory, Institute of Veterinary Sciences, University of Frères Mentouri Constantine 1, Constantine, Algeria.

2.5.1. Rose Bengal test (RBT)

The RBT is based on an antigen prepared from *B. abortus* S99 (smooth strain) stained with Rose Bengal dye and suspended in acid buffer (pH 3.65) to detect *Brucella* antibodies against *B. melitensis*, *B. abortus* and *B. suis* in serum. The test was conducted according to the fabricant's instructions (Lillidale Diagnostics ®, Dorset, United Kingdom) (Appendix 3). The principle of the test depends on an antigen-antibody reaction resulting in agglutination (Figure 10).

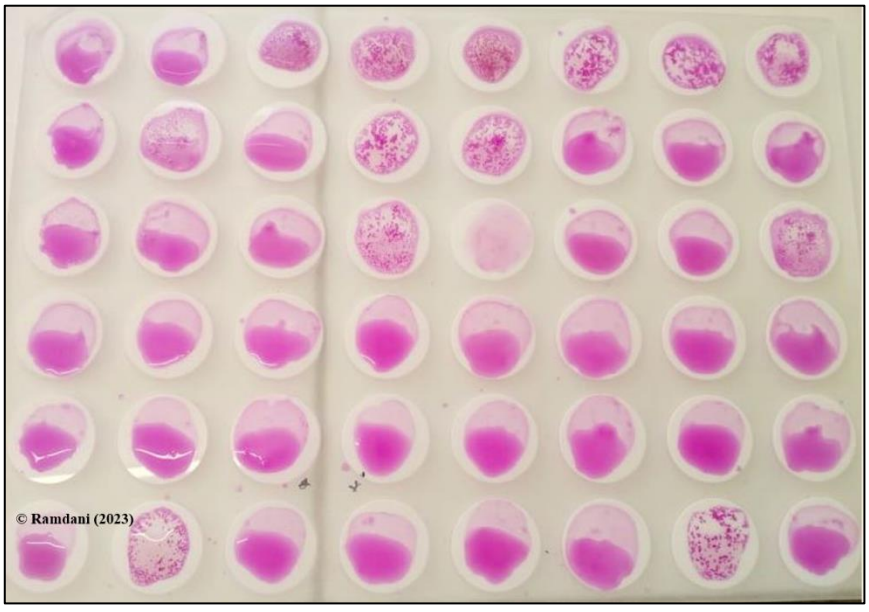

Figure 10: Rose Bengal plate test

2.5.2. Indirect enzyme-linked immunosorbent assay (iELISA) test

The indirect ELISA test is performed to detect antibodies against *B. abortus, B. melitensis* or *B. suis* by using a purified *Brucella* lipopolysaccharide (LPS) and a conjugate IgG anti-multispecies. The technique was conducted (Figure 11) according to manufacturer instructions (ID-VET®, Montpellier, France) (Appendix 4).

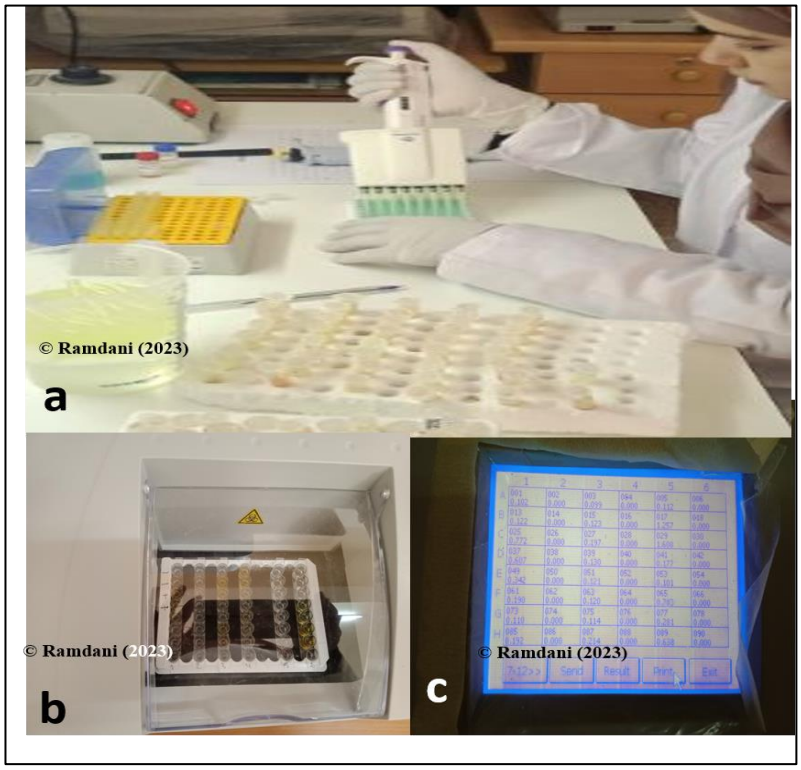

Figure 11: Steps for performing the indirect ELISA test in our study: a) Conduct of iELISA technique, b) Microplate reading via ELISA reader, c) Optical densities (OD) readings with the ELISA reader

2.5.3. Complement fixation test (CFT)

The complement fixation test allows the detection of anti-*Brucella* antibodies that are able to activate complement. In the first stage, heterologous complement is added to the antigen-serum mixture. In the presence of antibodies binding to the specific antigen, complements bind to these complexes (positive reaction). Subsequently, this invisible reaction is then revealed by the addition of haemolytic system (red cells-haemolytic serum).

The free complements bind to the said haemolytic system causing therefore lysis of erythrocytes. The hemolysis rate resulted from the latter reaction is inversely proportional to the antibodies titer in the sample (Figure 12).

CFT was performed according to the recommendation of the OIE (OIE, 2018) and the manufacturer instructions (ID-VET ®, Montpellier, France) (Appendix 5).

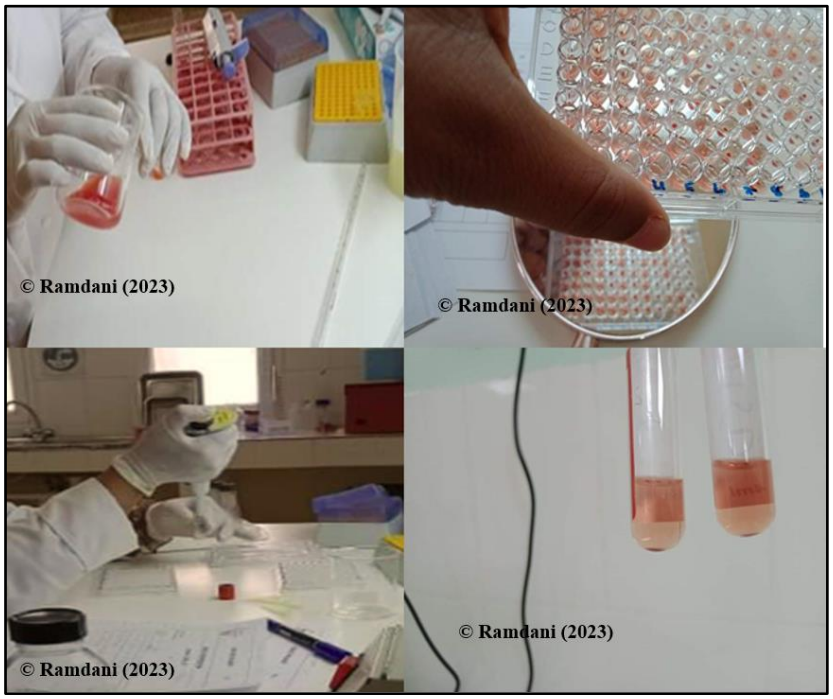

Figure 12: Procedure of complement fixation test

2.6. Data management and analysis

RBT and iELISA were used simultaneously. CFT was applied in series to both previous tests. Only serum samples that were positive to CFT were therefore considered positive. When at least one animal from the sampled herd tested positively, the herd was considered to be seropositive.

The true herd seroprevalence (HTP) and true individual prevalence (TP) were calculated according to the following formulas: $\mathrm{HTP} = \frac{\mathrm{HAP+HSPEC-1}}{\mathrm{HSENS+HSPEC-1}}$ and $\mathrm{TP} = \frac{\mathrm{AP+sp-1}}{\mathrm{se+sp-1}}$ respectively (Rogan and Gladen, 1978), using the online Epitools Calculator (Sergeant, 2018) (Figure 13). Where HAP is the herd apparent seroprevalence of brucellosis and AP is the individual apparent seroprevalence.

The abbreviations sp and se represent the specificity and sensitivity of the serological tests used for the detection of antibodies anti-*Brucella* spp. Blaker method was used to estimate 95% confidence intervals of the true prevalence as described by Reiczigel et al. (2010). HSPEC (Herd specificity) and HSENS (Herd sensitivity) were obtained from the outputs of FreeCalc application explained in sample size calculation section above.

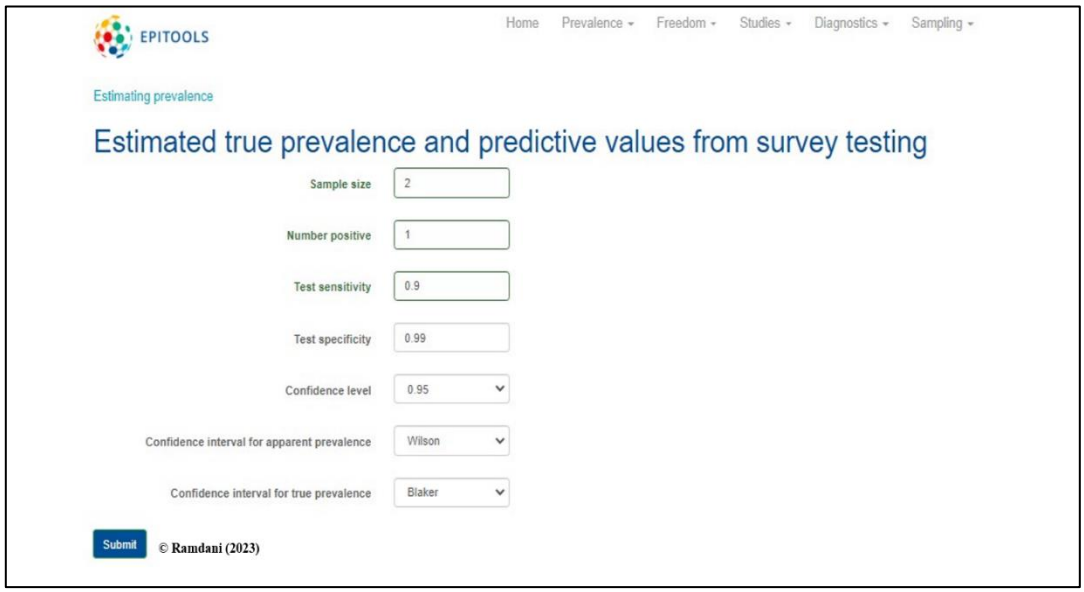

Figure 13: Calculation of true prevalence in Epitools website.

To show the spatial distribution of brucellosis prevalence in small ruminant herds by municipality in El Oued province, a choropleth map was created using ArcGIS 10.8.1 (ESRI, 2020).

The Epi info TM 7.2.3.0 (CDC, 2019) program was used to insert the questionnaires, which were then saved in Excel spreadsheet. Data were later cleaned and coded.

The association of each investigated variable to the status of herds regarding the seropositivity of *Brucella* spp. was determined by simple logistic regression.

Afterwards, *p* value ($p \leq 0.25$) and biological plausibility were used to determine which variables should be retained for the multivariable analysis. Cramer's V test was used to determine collinearity (V coefficient > 0.15) (Akoğlu, 2018). The collinear variable with the least biological plausibility was eliminated. Then, with a cut-off of 0.05 for entry and 0.1 for removal at each stage, a backward stepwise likelihood ratio test approach was used to carry out a binary logistic regression model. Statistically significant variables were those with a p-value < 0.05 at the final step.

Validation of the binary logistic regression model was determined by:

1) The Hosmer and Lemeshow test to assess the model's goodness of fit.
2) Identification of confounding factors if the change in log-odds when removing variables was greater than 20%.
3) Verification of significance of any interaction between the variables ($p < 0.05$).

IBM SPSS Statistics 25 was used to perform statistical analysis (IBM Corp., New York, USA).

3. Results

3.1. Seroprevalences of brucellosis

A total of fifty-one (51) herds was investigated. In each herd, twelve 12 animals were sampled to constitute six hundreds and twelve (612) goats and sheep (sheep = 280, goats = 332). Fourteen herds (14/51) contained one or more seropositive animals using RBT and iELISA for screening and CFT as a confirmatory test were recorded. The true herd prevalence was 27.95% (95% CI: 17.18–42.01).

At the individual level, thirty animals (30) (sheep and goats) were seropositive to the CFT, yielding a true individual seroprevalence of 3.98% (95% CI: 2.51–6.03).

3.2. Distribution of seroprevalence of brucellosis

Figure 15 shows the geographic distribution of *Brucella* spp. infection in small ruminant flocks by municipality. From the twenty-two (22) municipalities, nine (9) contained seropositive herds of small ruminant to brucellosis (Figure 14). The sampled small ruminant herds in three municipalities (Hamraia, Debila, and Magrane) showed 100% of seroprevalence. Whereas, half of the sampled flocks (50%) was demonstrated to be seropositive to *Brucella* spp. in El Oued and Taleb Larbi. Near the Tunisian border in Ben Guecha, there was a 40% frequency of brucellosis. The southernmost points of the province, Nakhla and Douar El-Ma, showed 33% and 20%, respectively, of *Brucella* spp. seroprevalence. Hassi khalifa displayed a seroprevalence of roughly 17%, while the remaining municipalities exhibited a complete lack of *Brucella* spp. seropositivity (See figure 14).

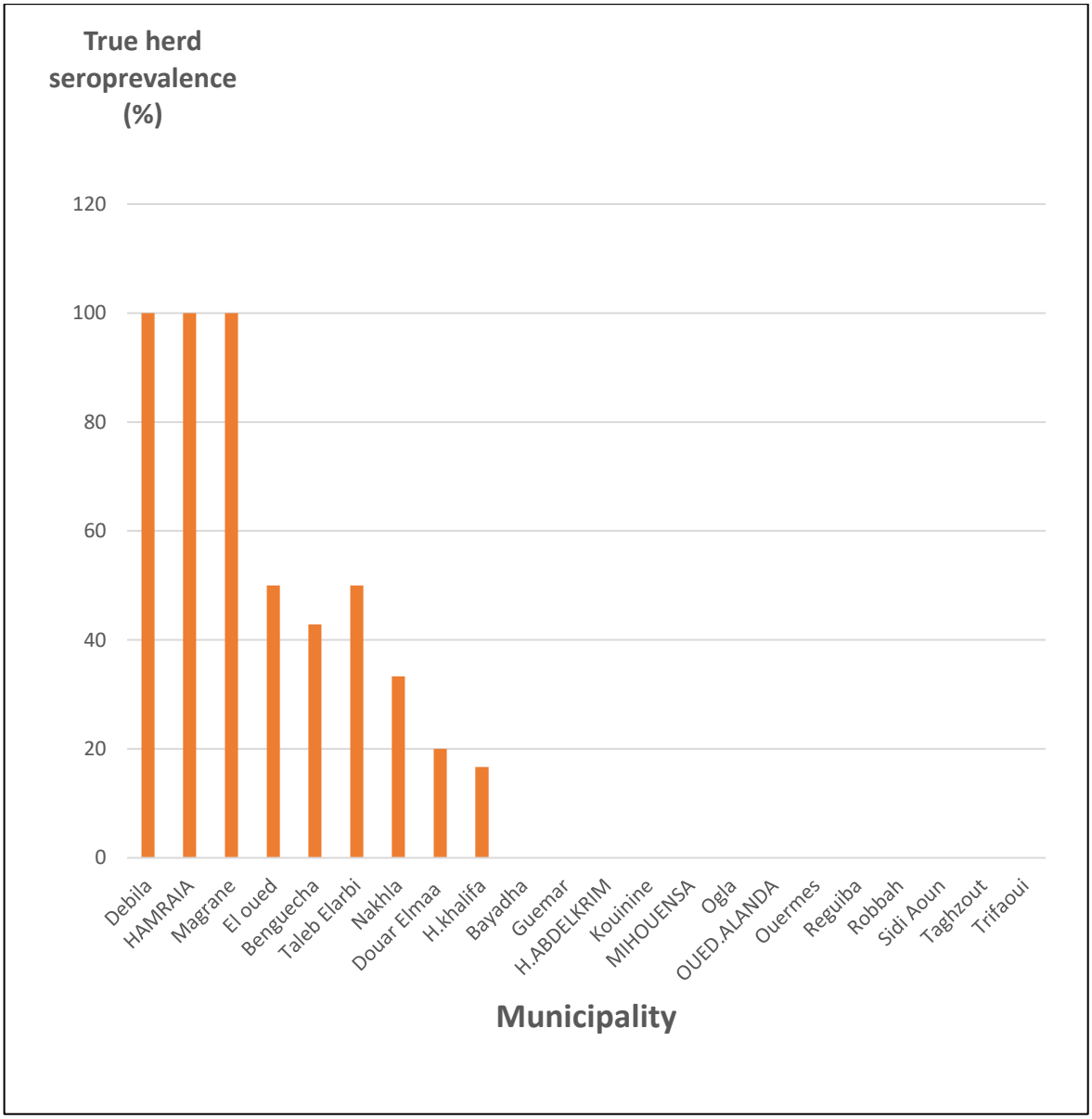

Figure 14: Column chart representing the true herd seroprevalence per municipality

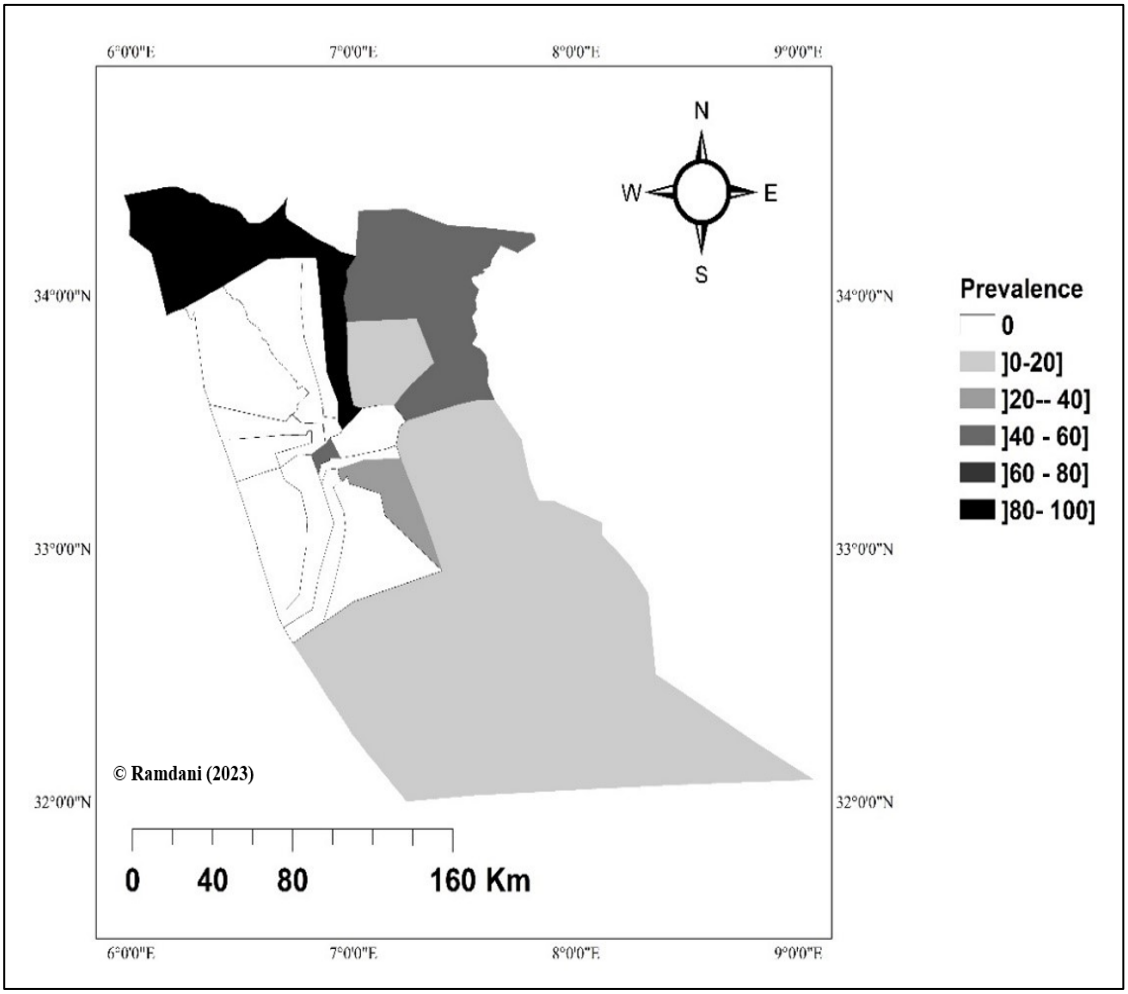

Figure 15: Choropleth map representing the distribution of the true prevalence of brucellosis in small ruminant herds per municipality in El Oued province, Algeria

3.3. Risk factors analysis

Three (3) variables: abortion history, poultry presence in the farm and herd owner work time, showed *p* value ≤0.25 at the univariate analysis (Table II), therefore, they were selected to be included into the multivariable analysis.

However, risk factors such as: herd size and composition, system and type of production, history of reproductive disorders such as retained placenta and stillbirth, contact with wildlife or other herds, keeping sheep and goat together, presence of other animals' species like cattle, camel, donkey and dog within the herd, purchasing new animals and self-reproduction, cleanliness of the livestock place, the frequency of the visits, the origin of the male reproducer and health management measures, among others, abortion, parturition and disease management, and quarantine of newly arrived animals, all appeared to be not significantly associated with the herd level brucellosis seropositivity (p>0.05) (Table II).

The univariate analysis revealed no significant difference between municipalities despite the major differences in seroprevalence from 100% to 0 (Table II).

Although the semi-extensive farming system showed a higher prevalence, no significant difference in the prevalence of brucellosis in small ruminant from different production systems (p > 0.05) was observed in our study (Table II).

Herds of small ruminant whose owner occupying other jobs disclosed a higher seroprevalence (40%) of brucellosis in comparison to flocks with full-time-herdsmen owner (15.4%). However, this difference was not statistically significant (p=0.06) (Table II).

Herds of different composition revealed varied seroprevalences despite the absence of significant difference (p=0.35%) (Table II).

Herds of size of less than 100 animals showed a seroprevalence of 31%, whereas herds with 100 animals or more revealed a seroprevalence of 22.7 %. Nonetheless, no significant difference was recorded in the univariate analysis (p=0.51) (Table II).

The production of farms whether it was commercial or traditional type disclosed similar seroprevalences (Table II). Accordingly, no statistical significance of difference in seroprevalence was revealed (p=0.91) (Table II).

The descriptive analysis revealed a considerable distinction of *Brucella* spp. seropositivity in regard of presence of multiple species such as camels, cattle, dogs, donkeys, and a mixture of sheep and goats within the herd of small ruminant (Table II). Nevertheless, no significant difference was observed (p>0.05) (Table II). With the exception of poultry's presence in herds that showed a significant difference (p=0.03). Their absence manifested a seroprevalence of 43.5% compared to their presence (14.3%).

Similarly, a disparity in seroprevalences was disclosed in herds with history of reproductive disorders like abortion, retained placenta and stillbirth as it is reported in Table II. However, only herds with history of abortion revealed a significant difference (p= 0.05) (Table II) in comparison to flocks with no problem of abortion in the last 12 months of the survey.

Herds that had contact with other herds and wildlife disclosed similar seroprevalences (around 27%) resulting in null differences (p>0.05) (Tale II).
A major distinction in seroprevalences in farms that adopted different methods for discarding abortion materials. Variation from 100% of brucellosis seropositivity in farms that burn the materials of abortion to 50% in herds that give it to dogs, whereas 28.6% and 13.3% of seroprevalences of brucellosis were recorded in farms that mange abortion martials by burying and public discharging, respectively (Table II). However, no significant difference was observed (p= 0.50) (Table II).

As a result of the univariate analysis, isolation of parturient and unhealthy animals has not resulted in any difference in the seropositivity of herds of small ruminant (p>0.05) (Table II).

Renewing the herd by self-reproduction resulted in the least seroprevalence (10%) in comparison to purchasing animals (29.6%), while adopting both methods brought the seroprevalence of herds about 35.7%. However, no significant difference was reported (p=0.4) (Table II).

The seroprevalence of herds that practiced quarantine of newly introduced animals (37%) was slightly higher than seroprevalence of herds that merged new animals with other animals without any delay (28), yet with no significant statistical difference(p=0.53) (Table II).

The seroprevalences associated to the frequency of cleaning variable varied greatly, from 22.2% to 100%. However, no difference statistically significant was observed (p=0.9) (Table II).

Forbidding visitors from entering the farm has not impacted the occurrence of the disease (p= 0.27) based on the univariate analysis (Table II).

The seroprevalence of brucellosis in herds that used outsider male for reproduction (32.1%) was slightly higher than those that used males raised within the herds for reproduction purposes (23.8%). However, no significant difference was recorded (p=0.52) (Table II).

Table II: Results of association between potential herd-level risk factors and small ruminant herd *Brucella* spp. seropositivity status at the univariate analysis

Variable	Category	No. positive/Total	Seroprevalence (%)	*P* value
Localization of the flock				
Municipality	Debila	1/1	100	1
	Hamraia	1/1	100	
	Magrane	3/3	100	
	El Oued	1/2	50	
	Ben Guecha	3/7	42.86	
	Taleb Elarbi	2/4	50	
	Nakhla	1/3	33.33	
	Douar Elmaa	1/5	20	
	Hassi khalifa	1/6	16.67	
	Bayadha	0/1	0	
	Guemar	0/1	0	
	Hassani Abdelkerim	0/1	0	
	Kouinine	0/1	0	
	Mih Ouensa	0/2	0	
	Ogla	0/2	0	
	Oued Alanda	0/1	0	
	Ouermes	0/1	0	
	Reguiba	0/3	0	
	Robbah	0/2	0	
	Sidi Aoun	0/1	0	
	Taghzout	0/1	0	
	Trifaoui	0/2	0	
Time employment of the owner				
Herd owner work time	Full time	4/26	15.4	0.06*
	Part time	10/25	40	
Characteristics and composition of the flock				
Production system	Intensive	10/40	25	0.59
	Semi-extensive	2/7	28.6	
	Agro-pastoral	2/4	50	
Herd composition (Mixed herd: animals from different origin)	Unified	12/39	30.8	0.35
	Mixed	2/12	16.7	
Herd size	<100	9/29	31	0.51
	≥100	5/22	22.7	
Production type (Commercial: bought to be re-put in the market for better price)	Traditional	13/47	27.7	0.91
	Commercial	1/4	25	
Camels	Yes	2/10	20	0.56
	No	12/41	29.3	
Cattle	Yes	1/6	16.7	0.54
	No	13/45	28.9	
Sheep only	Yes	1/2	50	0.48
	No	13/49	26.5	

Table II: Results of association between potential herd-level risk factors and small ruminant herd *Brucella* spp. seropositivity status at the univariate analysis (Continued)

Goats only	Yes	2/5	40	0.51
	No	12/46	26.1	
Sheep and goats	Yes	11/44	25	0.33
	No	3/7	42.9	
Dog	Yes	4/21	19	0.27
	No	10/30	33.3	
Poultry (pigeons and chicken)	Yes	4/28	14.3	0.03*
	No	10/23	43.5	
Donkey	Yes	1/4	25	0.91
	No	13/47	27.7	
Contact with other herds	Yes	4/15	26.7	0.94
	No	10/36	27.8	
Contact with wild animals	Yes	4/14	28.6	0.91
	No	10/37	27	
History of health issues				
Abortion **	Yes	10/24	41.7	0.05*
	No	4/25	16	
Retained placenta **	Yes	2/6	33.3	0.78
	No	12/43	27.9	
Stillbirth **	Yes	11/34	32.4	0.38
	No	3/15	20	
Management practices				
Abortion management **	Burn	2/2	100	0.50
	Burying	8/28	28.6	
	Public wasteland	2/15	13.3	
	Giving to dog	2/4	50	
Isolation of parturient **	Yes	5/15	33.3	0.63
	No	9/34	26.5	
Isolation of unhealthy animal	Yes	9/31	29	0.75
	No	5/20	25	
Renewing of herd (both: self-reproduction and purchase)	Self-reproduction	1/10	10	0.40
	Purchase	8/27	29.6	
	Both	5/14	35.7	
Quarantine of newly introduced animals ***	Yes	6/16	37.5	0.53
	No	7/25	28	
Cleaning frequency	Rarely	4/18	22.2	0.9
	Never	1/1	100	
	Frequently	9/32	28.1	
Frequent visitors	Yes	3/17	17.6	0.27
	No	11/34	32.4	
Reproducer male ** (Outsider: borrowed or purchased)	Outsider	9/28	32.1	0.52
	Raised	5/21	23.8	

* $P \leq 0.25$ were included into multivariate analysis.

** Herds containing only males for fattening purpose and temporary herds for commercial use (lacking reproduction history) were considered missing values.

*** Herds adopting self-reproduction (not concerned by quarantine) were considered missing values.

As a result of the final binary logistic regression model, history of abortion within the herd was revealed to rise (5) times the odds of *Brucella* spp. seropositivity in sheep and goats' herds (p=0.03) (OR: 6.25, 95% CI: 1.2–32.46) (Table III).

Additionally, it was disclosed that the presence of poultry in the flocks of sheep and goats (p=0.01) significantly decreased the incidence of seropositivity by 89% (OR: 0.11, 95% CI: 0.02-0.61). (Table III). The resulting model successfully fitted the data at df=6 (Hosmer and Lemeshow test: X2 = 2.92, p=0.82).

Table III: Binary logistic regression model results of factors associated to status of herds of small ruminant regarding seropositivity of *Brucella* spp.

Variable	Log-odds	Standard Error	Wald	Odds Ratio	95% Confidence Interval of Odds ratio	*P*-value
Abortion history	1.83	0.84	4.75	6.25	1.20-32.46	0.03
Poultry presence	-2.18	0.85	6.5	0.11	0.02-0.61	0.01

Model -2 Log likelihood: 43.33, X^2=15.3, p= 0.02

4. Discussion

4.1. Choice of serological tests

For the diagnosis of *Brucella* spp. infection in various animal species, a number of serological assays have been created (Nielsen and Yu, 2010). The inability of serological tests to identify all isotypes of antibody ani-*Brucella*, cross-reaction with other microorganisms, the prozone phenomenon, and the interference of vaccine antibodies all result in a reduction in performance. To ensure the best possible sensitivity and specificity, the present study has used a combination of assays. For the diagnosis of brucellosis in small ruminants, RBT and iELISA are particularly sensitive, but CFT is more specific but less sensitive than the prior tests (OIE, 2018). RBT and iELISA were thus carried out concurrently to boost sensitivity, both in serial testing with CFT.

Moreover, according to the World Organization for Animal Health (2018), RBT, iELISA, and CFT are the suggested techniques for determining the prevalence of brucellosis in a herd or flock.

Stournara et al. (2007) came to the conclusion that the majority of existing serological tests of diagnosis of *Brucella* spp. could be employed eleven (11) months after vaccination of sheep and goats without interfering with diagnostics. Consequently, the decision to use these serological tests was reinforced by the fact that Algeria has not vaccinated the livestock against brucellosis since 2017.

4.2. True herd seroprevalence

In comparison to estimates from earlier studies conducted in El-Bayafh (10.14%) (Nehari et al., 2014), Setif and Batna (15.84%) (Gabli et al., 2015), and nationally (3.33%) by Kardjadj et al. (2016), the true herd prevalence of brucellosis in small ruminants found in the current study (27.95%) was significantly higher. This major difference might be attributed to a number of elements: 1) the interruption of vaccination program even though the coverage rate was not considerable, 2) localization of the study area near the Tunisian border where the majority of livestock is located and adopting a transhumant grazing system, 3) the study's design, and 4) the choice of serological tests used for diagnosis of brucellosis.

To compare our findings to bordering countries, our prevalence estimate was comparable to rates found in Mali in small ruminants (25.2%) (Traoré et al., 2021) and in Tunisia in sheep (21.8%) (Barkallah et al., 2017). This similarity could be explained by the resemblance in livestock management method and lifestyle of the said countries. Furthermore, uncontrolled movement of animals through borders contributes to the transmission and persistence of infectious diseases.

Seroprevalence of brucellosis in Kuwait (89%) and in Jordan (34.3%) were considerably higher than our estimate (Musallam et al., 2015; Hegazy et al., 2016; Al-Sherida and al., 2020). Mussalam et al. (2015) attributed the high prevalence to insufficient control measures, unsupervised animal mobility, and a nomadic or itinerant farming method.

4.3. Distribution of seroprevalence of brucellosis in El Oued

According to expectations, the province of El Oued's northern and eastern municipalities, which share land borders with three other provinces (Tebessa, Khenchela, and Biskra), as well as Tunisia, reported the highest prevalence estimates. In this common land, livestock graze freely depending on the time of year and the availability of pasture, which increases the risk of disease transmission.

Additionally, roughly 35% of small ruminant flocks are concentrated in three municipalities close to the Tunisian border: Tableb Larbi, Benguecha, and Douar El-maa.

The high seroprevalence of brucellosis discovered in the Chief town municipality could be explained by the fact that El Oued area is known for being a commercial pole in Algeria, including the animal trade. Western areas had zero brucellosis seropositivity, which might be attributed to the region's higher use of young animal fattening farms and the absence of livestock transhumance as a result of the region's similar desert topography.

In low-budget and limited resources countries, the spatial distribution of brucellosis is gainful in terms of focusing on the most heavily infected areas regarding brucellosis control tools like test-and-slaughter, vaccination, livestock movement control, and awareness campaigns about farm biosecurity measures.

4.4. Risk factors associated to herds status of seropositivity

As a result of the multivariate analysis, the history of abortion revealed to be substantially correlated with the seropositivity status of the small ruminant herd. Despite the fact that brucellosis can affect both sexes, the most typical symptom of an acute infection is abortion in the final trimester in sheep and goats (Xavier et al., 2010). Abortion also contributes in survival of *Brucella* the flock, in addition to reducing production and causing infertility as a side effect. Undoubtedly, materials issued from abortion such as the placenta, fetuses, and fetal fluids are extremely infectious and serve as the primary means of transmission within the herd (Samadi et al., 2010). The findings of several researchers were consistent with our results (Boukary et al., 2013; Edao et al., 2020; Alemayehu et al., 2021).

Consequently, our results emphasize the relevance of establishing biosecurity measures on farms to decrease the incidence of brucellosis. In particular, abortion management measures such as burying abortion materials between two layers of quicklime, isolation and blood-testing aborted females and disinfection of livestock premises after abortion using common disinfectants such as phenol and chlorine.

Presence of chickens and pigeons within the flock of sheep and goats reduced the risk of seropositivity to *Brucella* spp. In this region, pigeons are raised as domesticated birds for their meat and eggs. Despite Roux's (1979) logical justification for the mechanical involvement of the aforementioned birds in the flock's transmission of brucellosis, poultry may also act as a natural insecticide by consuming pests. Blood-sucking insects, which feed on livestock’s blood, tears, and placental secretions, aid in the mechanical spread of brucellosis (Coelho et al., 2015).

Furthermore, *Brucella* was isolated from stomach contents of the stable fly (*Musca autumnalis*), *Ornithodoros*, and *Stomoxys calcitrans* (Coelho et al., 2015). Moreover, Huang et al. (2020) suspected that the recent outbreaks of brucellosis in Inner Mongolia might be ascribed to the rise of the population of ticks. This theory came as a result of isolation of *B. melitensis* biotype 3 from eggs and engorged adults of *Dermacentor nuttalli* (native tick species of Inner Mongolia). Other researchers (Jiang et al., 2019) found similar results, *Brucella* nucleic acid was detected in 19.74% of 1 084 ticks.

Also, *Brucella melitensis* nucleic acid was revealed in "engorged females-tick eggs" and “tick eggs-larvae” by the same researchers suggesting the possibility of transovarial and transstadial transmission of *B. melitensis* in ticks. Additionally, *B. melitensis* and *B. abortus* were discovered in *Dermacentor marginatus* ticks taken from sheep and cattle, respectively, while *Brucella* DNA was found in the eggs and larvae of engorged female ticks that were *Brucella* DNA-positive, revealing transovarial and transstadial transmission of *Brucella* spp. in *D. marginatus* ticks (Wang et al., 2018). Zhang et al. (2021) identified also *Brucella* spp. in *Haemaphysalis longicornis* ticks.

Taking into consideration these riveting findings, possibility of brucellosis to be a vector-born disease should be considered in the epidemiology of the disease. Indeed, contribution of ticks, fleas, or other blood-sucking insects in brucellosis infection should be taken into account in the region of study due to the decrease of the environmental transmission of *Brucella* spp. in arid areas. Consequently, chickens and pigeons in livestock premises may play a protective role as a vector control to diminish therefore the transmission of brucellosis.

Surprisingly, the prevalence of brucellosis was not found to be correlated with putative risk factors in the current investigation, including pastoralism, commercial production type, herd size, using purchased animals for renewal, retained placentas and stillbirth histories, and provenance of the breeding male. The significant discrepancy between the estimated true prevalence (27.95%) and the expected true prevalence (3.33%), for example, may have skewed the sample size and contributed to the lack of statistical relationship. Along with the subjectivity of some data gathered in the questionnaire, bias in cross-sectional research linked to selection and confounding, as stated by Pandis (2014), may also be a factor.

5. Conclusion

This study is the first in Algeria where several serological tests have been used for the diagnosis of brucellosis in sheep and goats. In conclusion, this study reveals the high seroprevalence of small ruminant brucellosis in southeastern Algeria and indicates that the seropositive herds were located in bordering areas with Tunisia and regions with higher livestock density. In addition, our study indicates that history of abortion in herds of small ruminant increased substantially the likelihood of brucellosis and that presence of chickens and pigeons in livestock barns reduced considerably the occurrence of the disease.

Based on the true herd prevalence estimate, the mass vaccination strategy of the whole flock is highly recommended in order to mitigate contamination, abortion rate and transmission of the disease, targeting specifically the highest prevalence areas. In addition, our findings highlight the importance of the implementation of biosecurity measures at farms to diminish the risk of introduction, establishment and spread of this transboundary disease. Therefore, education programs for farmers and farm workers to implement effective biosecurity measures are highly required. Moreover, insect control should be added to the control/eradication plan of the disease due the possibility of vector transmission of the brucellosis.

Chapter 2

Modeling of Brucellosis in Small Ruminant in El Oued Province

1. Problem statement and specific objectives

Different strategies have been suggested by many authors regarding short/long term control or eradication programs of brucellosis. However, Benkirane (2006) and Minas (2006) agreed on three main consecutive strategies: mass vaccination, vaccination of young small ruminants combined with test-and-slaughter of adults and finally test and slaughter of adults only, depending on the prevalence from the highest level to the lowest, respectively. Yet, brucellosis is still prevalent in many parts of Algeria despite the time and monetary costs for different control programs. Meanwhile, mathematical modeling and simulation of infectious diseases prevent unavailing control programs. Therefore, simulation of different scenarios of control approaches in order to plump for the most effective strategy is highly required particularly in low and middle-income countries due to limited resources. In this context, a dynamic transmission model of small ruminant brucellosis and deterministic simulation models of different control scenarios related to the three control strategies described earlier were developed in El Oued province, in the southeast of Algeria. The aim of the study was to demonstrate the impact of different simulated control programs over 20 years on the seroprevalence of brucellosis in small ruminant population and reveal the most effective policy.

2. Materials and methods

Two types of models were developed: a deterministic mathematical model of transmission dynamics of brucellosis in small ruminant in El Oued province (Algeria) using compartmental model to represent the various categories of the animal population related to the studied disease, and a deterministic simulation agent-based-model (ABM) including fixed set of inputs, essential components of the proposed control strategies in order to simulate the various outcomes over twenty (20) years.

2.1. Definition of essential data for modeling and simulation

2.1.1. Animal population in the study area

Sheep and goats in the study area are composed of a mixture of breeds. Data related to phenotypic characterization of goat breeds in Algeria are poorly documented.

Therefore, collected figures regarding the average longevity in local breeds, prolificacy, number of births a year and parturition rate used to model the dynamics of transmission of brucellosis were related to ovine species (Table V).

The slaughtered animals (C) each year are mostly fattening young animals. Thus, they do not have any role in transmission of the disease because they are mostly susceptible and unable to transmit the disease.

Birth rate (b) was calculated according to the formulae: b= New births/ Population of small ruminant (N)= (Number of adult females × parturition rate× Prolificacy× Number of births a year)/N.

Death rate (μ) was calculated as follows: 1/ Life expectancy.

2.1.2. Epidemiological data

The study area is an endemic region in brucellosis. The true individual prevalence in this area was estimated by 3.98% (Ramdani et al., 2022). The control program adopted in the area was based on vaccination of small ruminant from 2010 to 2017. Seropositive animals investigated after reporting human cases are subjected to slaughtering. The proportion of slaughtered reactors is too small compared to the proportion of infected animals.

2.1.3. *Brucella melitensis* and brucellosis in small ruminant

B. melitensis is the main cause of brucellosis in small ruminants. Latent infections by *B. melitensis* acquired *in utero* in small ruminant kids are less frequent (Grillo et al., 1997). Therefore, the slaughtered young animals would be part of the susceptible population in our model.

Brucellosis is a chronic infection due to prolongation of cells life by apoptosis inhibition (Moreno and Gorvel, 2005; He et al., 2006). Additionally, strains of *B. melitensis* were detected in vaginal discharges up to 68 days post-abortion and 44 days post-partum. However, it appeared to be continuously excreted in milk of ewes (Tittarelli et al., 2005). Therefore, the infectiousness of infected animals seemed to last until death.

According to Olsen et al. (2010), environmental persistence of *Brucella* is epidemiologically insignificant because close contact is indispensable for transmission due to transience of environmental contamination. For that reason, environmental contamination was neglected in our model.

2.1.4. Brucellosis vaccines

The most available vaccine used in small ruminant is a live attenuated smooth (S) *Brucella melitensis* Rev1 (Blasco and Molina-Flores, 2011). It has proven to be the most effective vaccine (Barrio et al., 2009). The duration of immunity conferred by Rev1 vaccine was found to last 4.5 years in goats (Alton, 1968) and 2.5 years in sheep (Alton, 1990). To avoid overestimation of immunized animals, wanning immunity post-vaccine in our model was calculated based on conferred immunity in sheep.

2.2. Models' formulation

2.2.1. Deterministic SI model for transmission dynamics of brucellosis in small ruminant

A deterministic SI mathematical model for the transmission dynamics of brucellosis is formulated. The population of small ruminants (N) is composed of two compartments which are the susceptible (S) and infected (I) subpopulations. Young animals of replacement rate (α_1) that consisted of new born (b(N)) minus the slaughtered young fattening animals (c (S)) $\alpha_1 =$ b (N) $-$ c(S) are added to the susceptible subpopulation per year $^{-1}$. Susceptible animals become infected at the transmission rate ß after an effective and direct contact with infectious individuals SI per year $^{-1}$. Infected animals are assumed to be infectious after contracting the infection. The infectiousness is assumed to last during the lifespan of the animal. The death rate (µ) is assumed to be natural death. The total population of small ruminants (N) was assumed to be homogenously mixed. The flow diagram (Figure 16) describes the dynamic of transmission of brucellosis model in small ruminants.

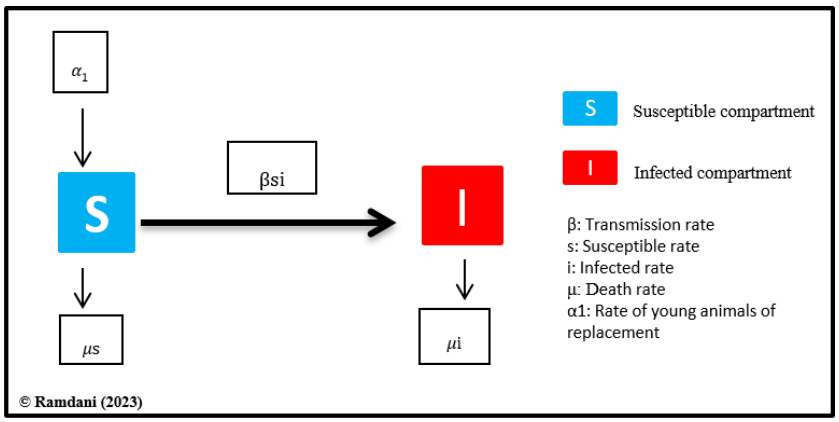

Figure 16: Flow diagram of the dynamic of transmission of brucellosis in small ruminant

The model was developed using ordinary differential equations (ODE) for each time *t* as follows:

$\frac{ds}{dt} = \alpha_1 - \beta si - \mu s$ (1)

$\frac{di}{dt} = \beta si - \mu i$ (2)

N= S+I (3)

s +i=1 (4)

s=S/N, i= I/N.

2.2.1.1. Disease free equilibrium

At the disease-free equilibrium point x_0 (DFE), the model (S, I) = (s(x_0), 0). Solving the equation (1) after setting S'=0, the model (S(x_0), 0) = ($\frac{\alpha_1}{\mu}$, 0).

The basic reproduction number R0 was computed using the next generation matrix method (Van den Driessche and Watmough, 2002) as described below:

At DFE x_0, we have:

$F = \frac{dF}{dI} = ßs = \frac{ß\alpha_1}{\mu}$

$V = \frac{dV}{dI} = \mu$

F represents the new infections in compartment I, while the V represents the rate of transfers of infections from compartment I to other compartments or transitions between compartment I and other infected compartments (Van den Driessche, 2017).

FV^{-1} = R0, therefore the basic reproduction number is given by:

$$R0 = \frac{\beta\alpha_1}{\mu^2}$$

2.2.1.2. Endemic equilibrium state

The study area is an endemic region in brucellosis. Therefore, the transmission of brucellosis in small ruminants is assumed to occur at an endemic equilibrium (EE).

At the point x_e (DEE) of the model, the effective reproduction number R(t) would be equal to 1 (Miller, 2003).

We have $R(t) = \frac{S(t)}{N} R_0$ and $R(t) \leq R_0$ (Cintrón-Arias et al., 2009).

$$R(t) = \frac{S(t)}{N} \times \frac{\beta \alpha_1}{\mu^2}$$

At the DEE point, the transmission rate is a time and density dependent parameter that would be given by:

$$ß(t) = N \frac{\mu^2}{\alpha_1 S(t)} \quad \text{Or} \quad ß(t) = \frac{\mu^2}{\alpha_1 s(t)}$$

The model was simulated over twenty years (20) without any control interventions.

2.2.2. Deterministic simulation models of control strategies

Deterministic simulation agent-based-model (ABM) models were developed over 20 years. The initial starting point would be the endemic equilibrium state where the infected proportion at (t_0) is the true prevalence of brucellosis in small ruminant (3.98%) (Ramdani et al., 2022). Three control strategies are simulated and the output parameter is the infected proportion of animals which is the indicator of outcomes of simulated control policies.

2.2.2.1. Simulation of vaccination only

Vaccination with Rev 1 vaccine of small ruminant is simulated over 20 years. A new compartment of subpopulation of vaccinated animals (V) is added to the dynamic transmission model. The proportion of animals to be vaccinated per year $^{-1}$ is ɣ. The efficacy of Rev1 vaccine is ε. The waning immunity rate of vaccine σ: 1/ duration of immunity due to Rev1 vaccine in small ruminant. The adult animals (α_2) non-infected and non-vaccinated are part of the susceptible subpopulation $\alpha_2 = S - \alpha_1$. Three-vaccination deterministic simulation models were formulated depending on the category of animals vaccinated (All susceptible animals, young animals only and adult animals only). Therefore, only susceptible and vaccinated compartments change accordingly. The structure of the model is demonstrated in Figure 17.

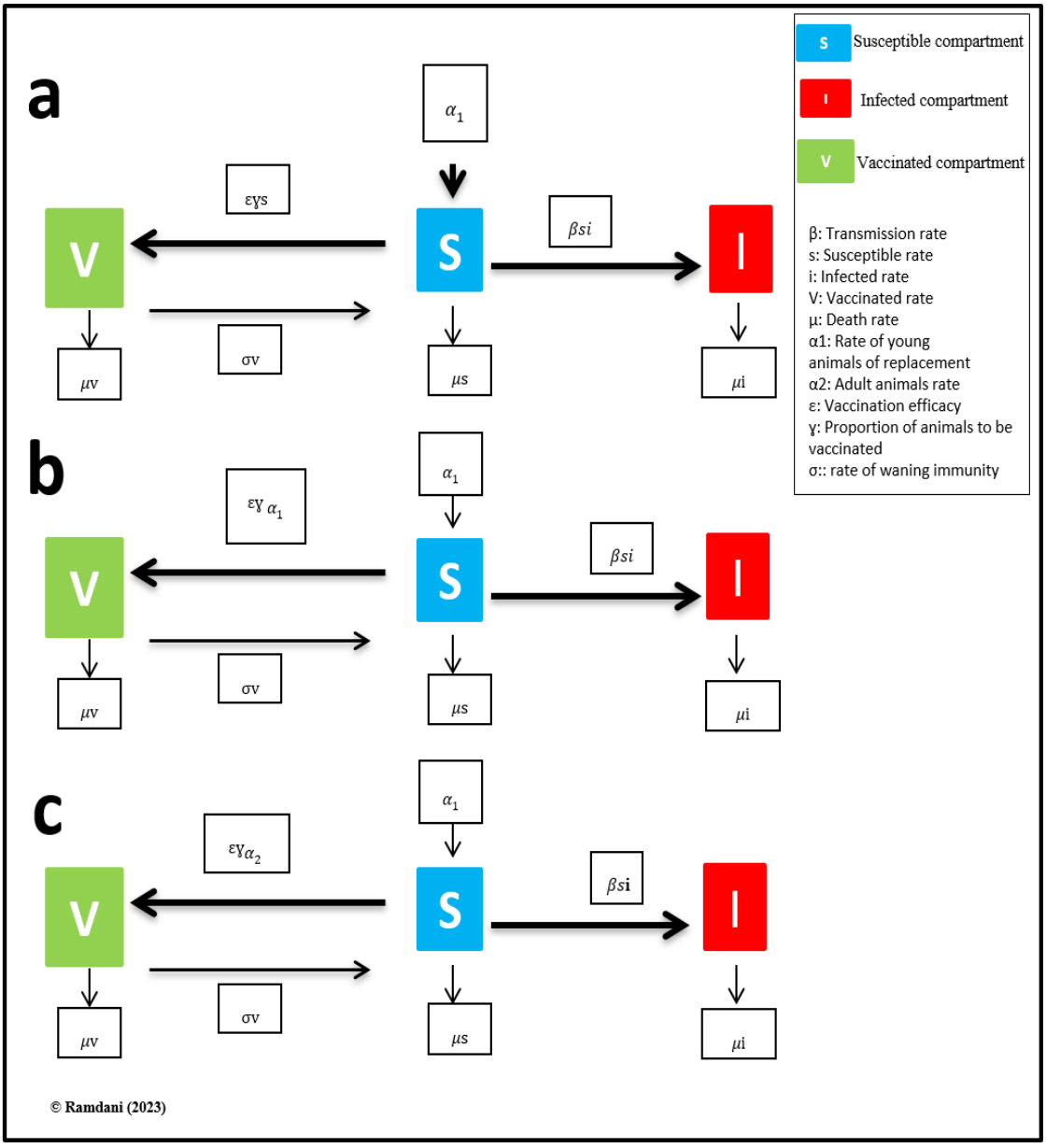

Figure 17: Flow diagram of the dynamic of transmission of brucellosis in small ruminant. Including vaccination of young and adults (a), young only (b) and adult only(c)

The mathematical structure of the model simulating the mass vaccination MV (young and adult) is given by the following ODE:

$$\frac{ds}{dt} = \alpha_1 + \sigma v - \beta si - \mu s - \varepsilon \gamma s \qquad (7)$$

$$\frac{di}{dt} = \beta si - \mu i \qquad (8)$$

$$\frac{dv}{dt} = \varepsilon \gamma s - \sigma v - \mu v \qquad (9)$$

Simulation of vaccination of young animals (VY) only is given by the structure:

$$\frac{ds}{dt} = \alpha_1 + \sigma v - \beta si - \mu s - \varepsilon\gamma\alpha_1 \qquad (10)$$

$$\frac{dV}{dt} = \varepsilon\gamma\alpha_1 - \sigma v - \mu v \qquad (11)$$

Simulation of vaccination of adult animals (VA) only is given by the following ODEs:

$$\frac{ds}{dt} = \alpha_1 + \sigma v - \beta si - \mu s - \varepsilon\gamma\alpha_2 \qquad (12)$$

$$\frac{dv}{dt} = \varepsilon\gamma\alpha_2 - \sigma v - \mu v \qquad (13)$$

2.2.2.2. Simulation of vaccination combined with test-and-slaughter (VTS) policy

This model consisted of simulating the vaccination of young animals and test-and-slaughter of adults. The proportion of animals to be sampled per year $^{-1}$ is (κ).

Rose Bengal test (RBT) was suggested to be used for screening and Complement fixation test (CFT) as confirmatory test. RBT and CFT are the recommended techniques by the World Organisation for Animal Health (2022) in implementing eradication policies. However, these serological tests are not perfect. The sensitivity (Se_b) and specificity (Sp_b) of both tests applied in series are calculated as described by Thrusfield (2007):

$$Se_b = Se(RBT) \times Se(CFT)$$

$$Sp_b = 1 - [(1 - Sp(RBT)) \text{ x } (1 - Sp(CFT))]$$

In consequence, animals tested seropositive to both tests should be eliminated by slaughtering.

Thus, reactors might be true positive ($Se_b \times i$) or false positive $[(1 - Sp_b) \times \alpha_2]$. The model is illustrated in Figure 18.

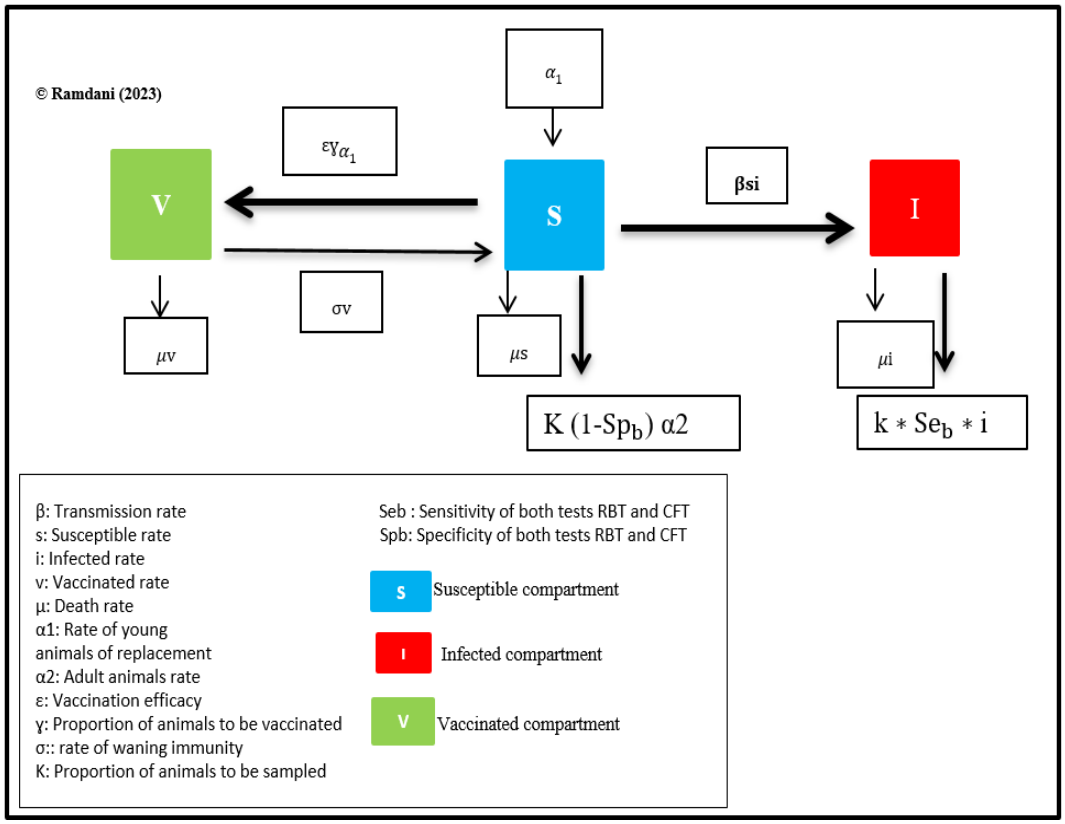

Figure 18: Flow chart of the dynamic of transmission of brucellosis including test-and-slaughter policy combined with vaccination

The model is represented by:

$$\frac{ds}{dt} = \alpha_1 + \sigma v - \beta si - \mu s - (\varepsilon\gamma \times \alpha_1) - [\kappa \times (1 - Sp_b) \times \alpha_2] \quad (14)$$

$$\frac{di}{dt} = \beta si - (\kappa \times Se_b \times i) - \mu i \quad (15)$$

$$\frac{dv}{dt} = \varepsilon\gamma\alpha_1 - \sigma v - \mu v \quad (11)$$

2.2.2.3. Simulation of test-and-slaughter (TS) strategy only

Serological tests have no practical diagnostic significance on young animals (Tittarelli et al., 2005). Therefore, only adults shall be sampled at the proportion κ. The structure of the model is described in Figure 19.

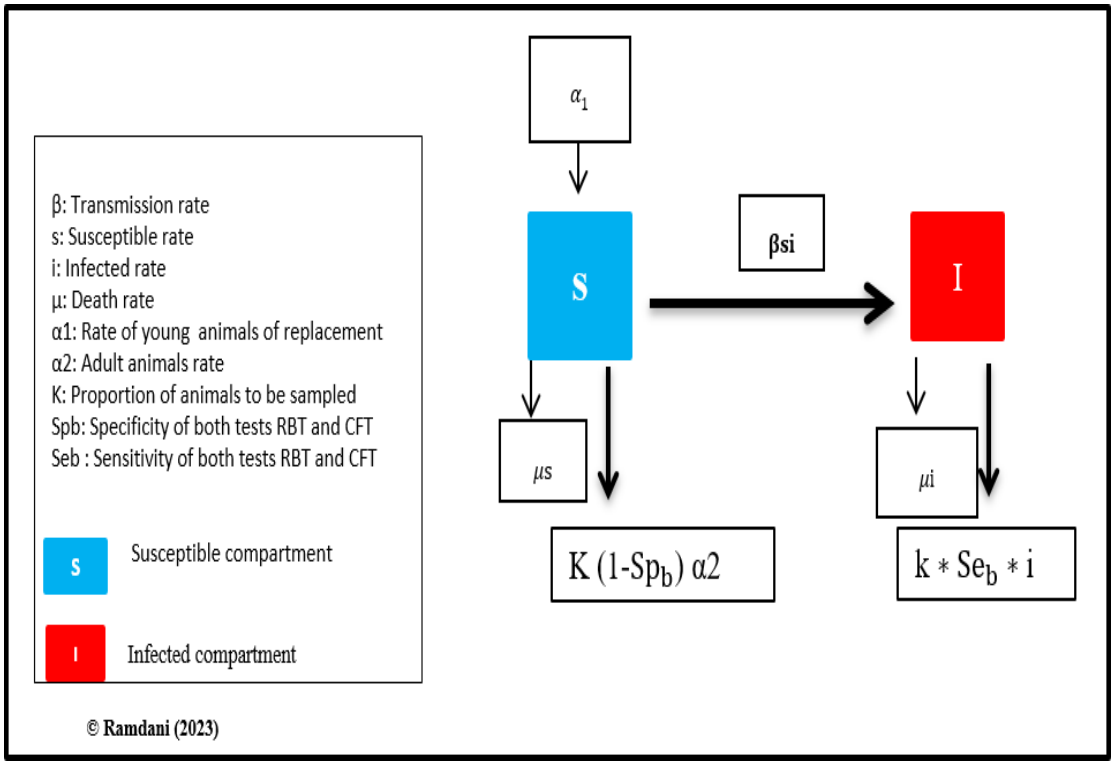

Figure 19: Flow chart of the dynamic of transmission of brucellosis including test-and-slaughter of adult policy

The model is represented mathematically as:

$$\frac{ds}{dt} = \alpha_1 - \beta si - \mu s - [\kappa \times (1 - Sp_b)\alpha_2] \quad (16)$$

$$\frac{di}{dt} = \beta si - (\kappa \times Se_b \times i) - \mu i \quad (15)$$

The input parameters of all models formulated above are summarized in Table IV. The essential values for calculation of the input parameters are described in Table V.

Table IV: Input parameters used in mathematical models

Input parameters	Symbol	Value	References
Susceptible proportion-endemic stability	s(0)	0.9602	Ramdani et al. (2022)
Infectious proportion-endemic stability	i(0)	0.0398	Ramdani et al. (2022)
Transmission rate	B	$ß = \frac{\mu^2}{\alpha_1 s(t)}$	Time and density dependent variable calculated at endemic equilibrium point
Death rate	μ	0,092	Calculated
Proportion of young animals of replacement	α_1	b-cs(t)	Density dependent parameter Calculated
Proportion of susceptible adult animals	α_2	$s - \alpha_1$	Density dependent parameter Calculated
Sensitivity of RBT and CFT in series	Se_b	61.09%	Calculated
Specificity of RBT and CFT in series	Sp_b	99.99%	Calculated
Proportion of animals to be sampled	K	5%, 10%, 15%, 20%, 30% 50%, 75%	Fixed values depending on the control policy
Vaccination efficacy	E	100%	Barrio et al. (2009)
Proportion of animals to be vaccinated per $year^{-1}$	ɣ	20%, 25%, 50%, 75%, 90%, 100%	Fixed values depending on the control policy
Time step	Dt	0.0027 (one day)	Assumed
Maximum time	Tmax	20 years	Assumed
Waning immunity post-vaccination	Σ	0.4	Calculated

Table V: Essential values used to calculate the input parameters of the models

Parameter	Values	References
Duration of immunity due to Rev1 vaccine	2.5 years	Alton (1990)
Birth rate(b)	0.551	Calculated
Slaughtering rate of fattening young animals (c)	0.3588	DSA El Oued (2021)
Sensitivity (RBT)	75.8%	Minas et al. (2008)
Specificity (RBT)	99.7%	
Sensitivity (CFT)	80.6%	
Specificity (CFT)	99.1%	
Proportion of adult caprine females	0.54	DSA El Oued (2021)
Proportion of adult ovine females	0.43	DSA El Oued (2021)
Total population of small ruminant number in El Oued (N)	N= 980680	DSA El Oued (2020)
Prolificacy	1.25	Chekkal et al. (2015)
Parturition rate	93.83%	Benyoucef et al. (2000)
Number of births a year	1	Chekkal et al. (2015)
Life expectancy	10.86	Chekkal et al. (2015)

2.3. Models' running and data analysis

Thirty-two (32) models including the null model (without any intervention) were run separately. We used a time step (dt) of one day. The models were coded and run in MATLAB version 9.8.0 (The Mathworks Inc, Massachusetts, USA).

We used Euler's method (Earn, 2008) to simulate our models for s(it), i(it) and v(it) as follows:

s(it) = s(it-1) + ds

i(it) = i(it-1) + di

v(it) = v(it-1) + dv

Figures of line graphs were created using Origin (Pro), version 9.9.0.225 (Origin Lab Corporation, Massachusetts, USA). Bar graphs were created using Microsoft Excel.

3. Results

The values of infected, susceptible and vaccinated proportions obtained at 5, 10 and 20 years using mathematical modeling of transmission dynamic of brucellosis in small ruminant without intervention and after simulation of thirty-one (31) control strategies are summarized in appendix 6.

Simulating the dynamic of transmission of brucellosis in small ruminant over the next 20 years without any intervention revealed a slight decrease of the prevalence from 3.98% to 3.09%, 2.40% and 1.47% after 5 years, 10 years and 20 years, respectively (Appendix 6, Figures 20 and 21).

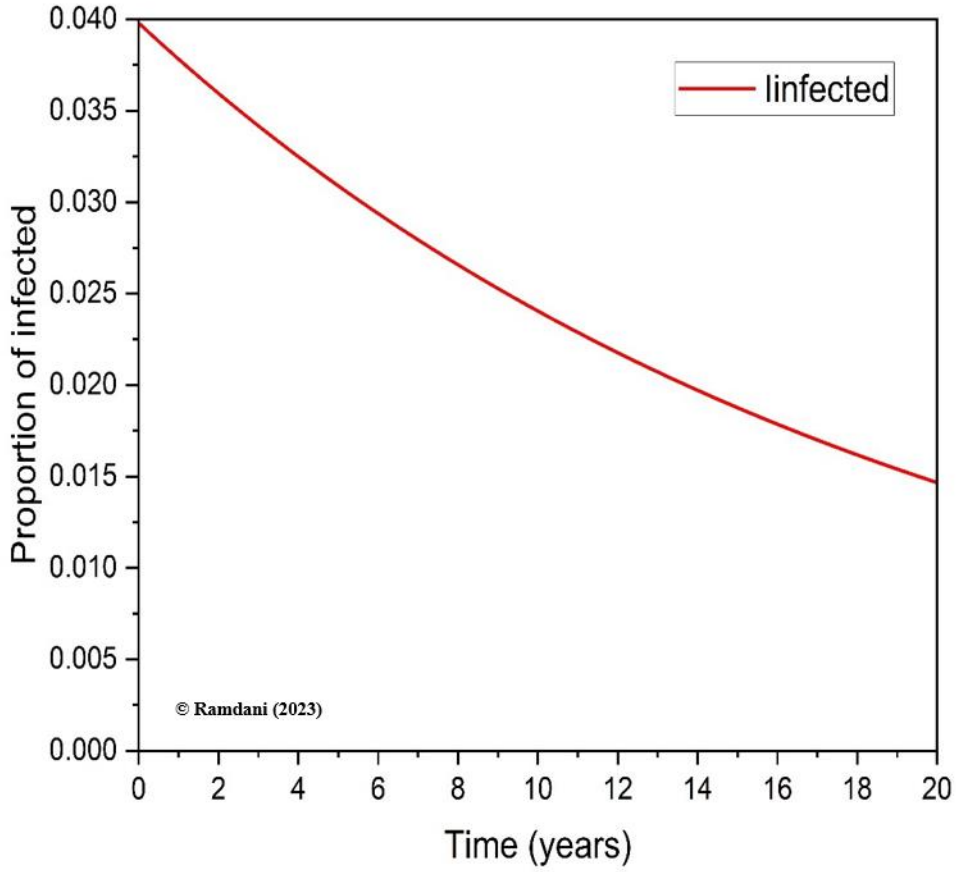

Figure 20:Simulation of dynamic of brucellosis without intervention in small ruminant over 20 years

The figure 20 represents a line graph that illustrates the prediction of the dynamic of the spread of the disease in small ruminant for the upcoming 20 years via the infected rate which is the indicator of the disease.

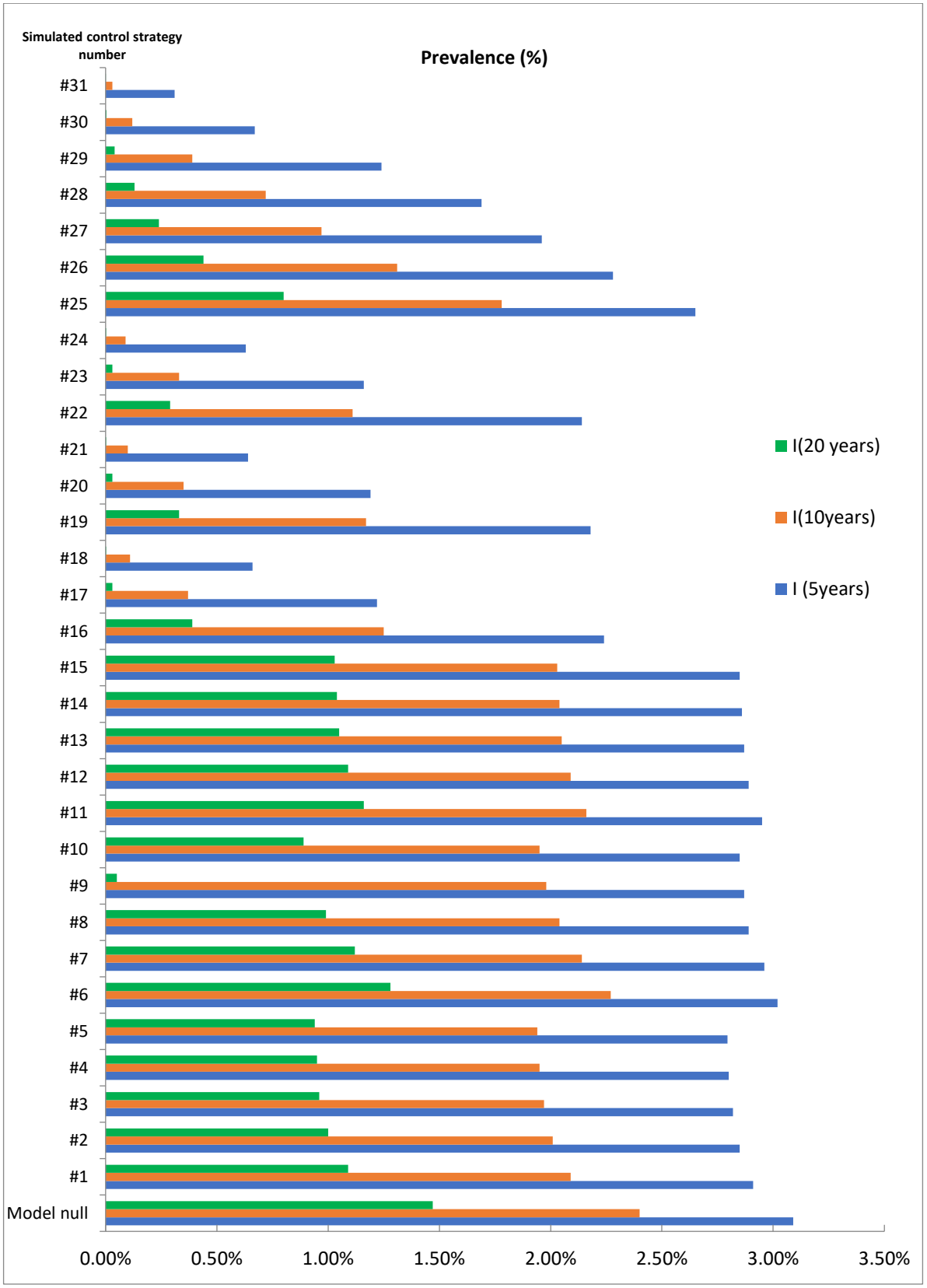

Figure 21: Brucellosis prevalence in each simulated control strategy in 5, 10 and 20 years

The figure 21 represents a bar chart that showcases the results as the prevalences of all simulated control/eradication policies as well as the deterministic model of the dynamic transmission of brucellosis after 5,10 and 20 years. The strategies are coded from #1 to #31. The codes are explained in appendix 6.

Simulation of vaccination policy of 25%, 50%, 75%, 90% and 100% of all categories of animals (young and adult) over 20 years resulted in the prevalence estimates: 1.09%, 1%, 0.096%, 0.095% and 0.094%, respectively as shown in appendix 6 and figures 21 and 22.

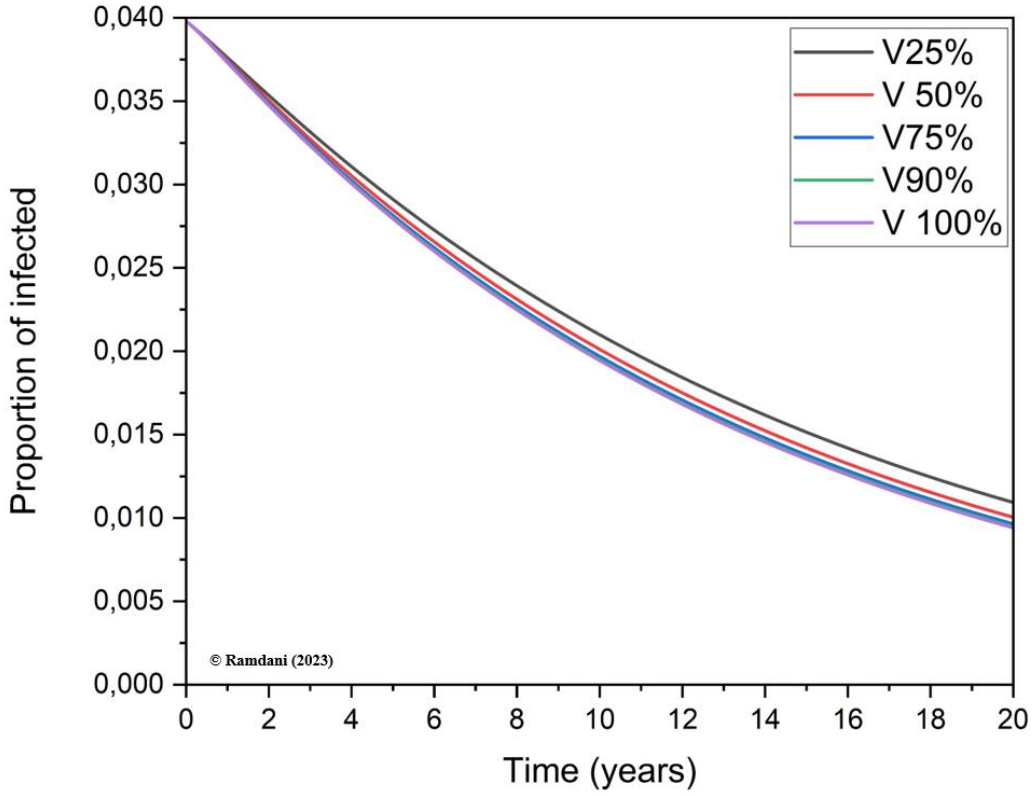

Figure 22: Simulation of dynamic of brucellosis in small ruminant after mass vaccination (V) for 20 years

Figure 22 represents a line graph that manifests the proportion of infected animals for the upcoming 20 years after implementing vaccination of 25%, 50%, 75%, 90% and 100% of the whole population of small ruminant in El Oued.

Simulation of vaccination policy of 25%, 50%, 75%, 90% and 100% of only young small ruminant over 20 years resulted in the prevalence estimates: 1.28%, 1.12%, 0.99%, 0.05% and 0.89%, respectively as demonstrated in appendix 6 and figures 23, and 21.

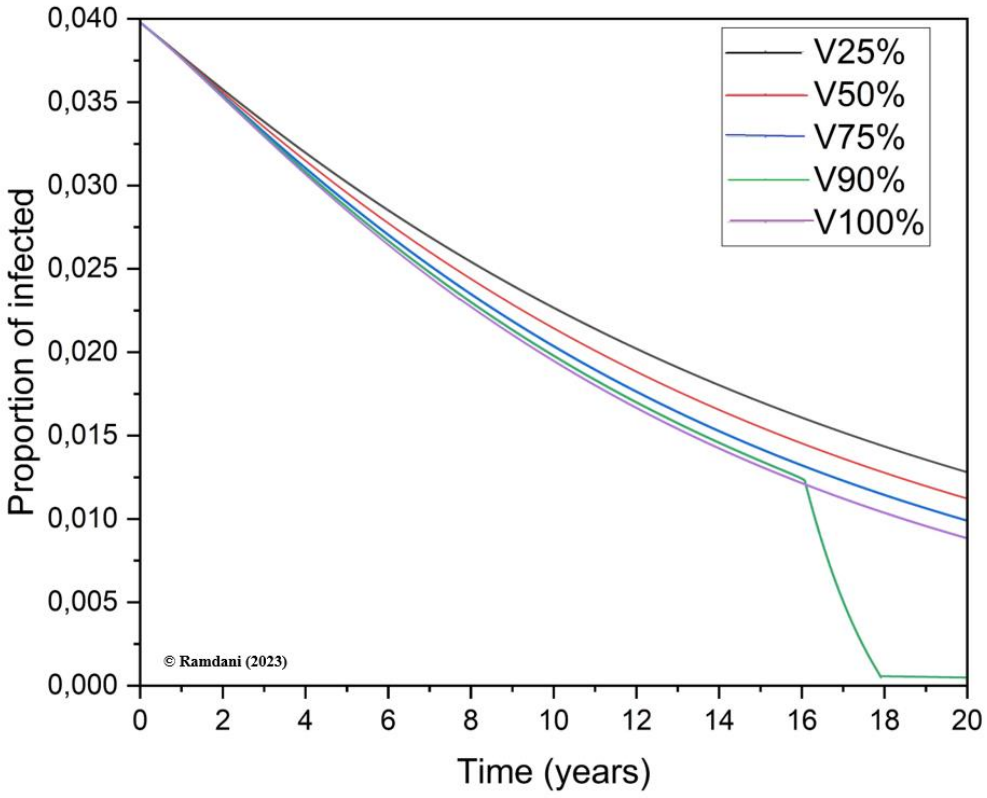

Figure 23: Simulation of dynamic of brucellosis in small ruminant after young vaccination (V) for 20 years

The figure 23 represents a line graph that reports the proportion of infected animals after application of vaccination of 25%, 50%, 75%, 90% and 100% of young animals of sheep and goats over the next 20 years.

Simulation of vaccination policy of 25%, 50%, 75%, 90% and 100% of only adult animals over 20 years resulted in a gradual reduction of the prevalence estimate as follows: 1.16%, 1.09%, 1.05%, 1.04% and 1.03%. Appendix 6 and figures 21 and 24 illustrate further the results of the adult vaccination policy.

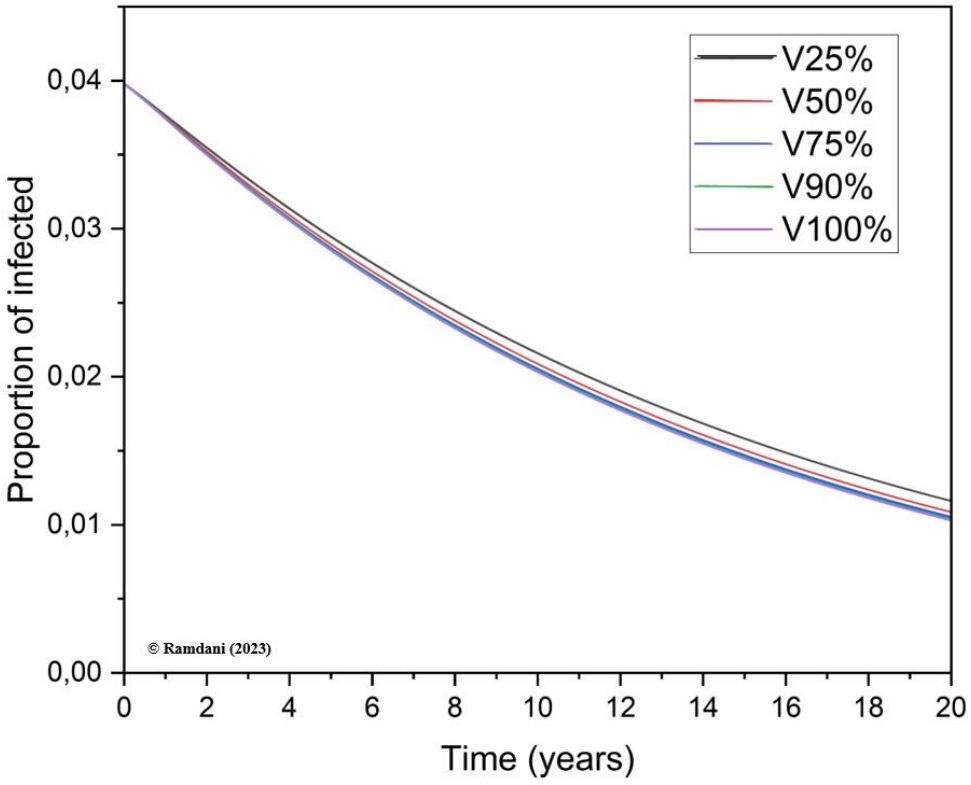

Figure 24: Simulation of dynamic of brucellosis in small ruminant after adult vaccination (V) for 20 years

The figure 24 represents a line graph that shows the proportion of infected animals after implementing vaccination of 25%, 50%, 75%, 90% and 100% of adult animals over the next 20 years.

Forecasting the combination of vaccination of 20 % of young animals and test-and-slaughtering of 10%, 30% and 50% of adults over 20 years brought the prevalence of brucellosis to: 0.39%, 0.03% and 0%, respectively. Whereas, vaccination of 50 % young animals and test-and-slaughtering of 10%, 30% and 50% of adults over 20 years brought the prevalence estimates down to: 0.33%, 0.03% and 0%, respectively. While vaccinating 75 % of young animals and testing of 10%, 30% and 50% of adults for elimination over 20 years resulted in the following prevalence estimates: 0.29%, 0.03% and 0%, respectively. Appendix 6 and figures 21, 25, 26 and 27 demonstrate the results of these simulation models.

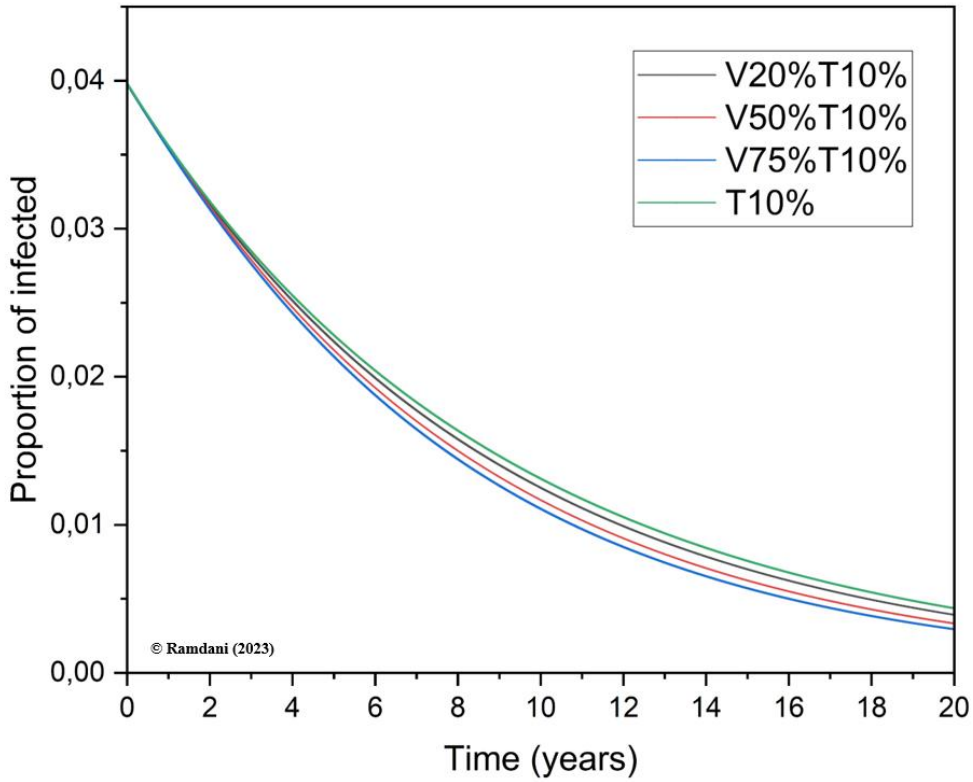

Figure 25: Simulation of dynamic of brucellosis in small ruminant after 10% of test-and-slaughter (T) alone and combined with vaccination (V) for 20 years

The figure 25 represents a line graph that reveals the proportion of infected animals after application of policies based on test-and-slaughter of 10% of adults alone and combined with vaccination of 20%, 50% and 75% of young animals over the upcoming 20 years.

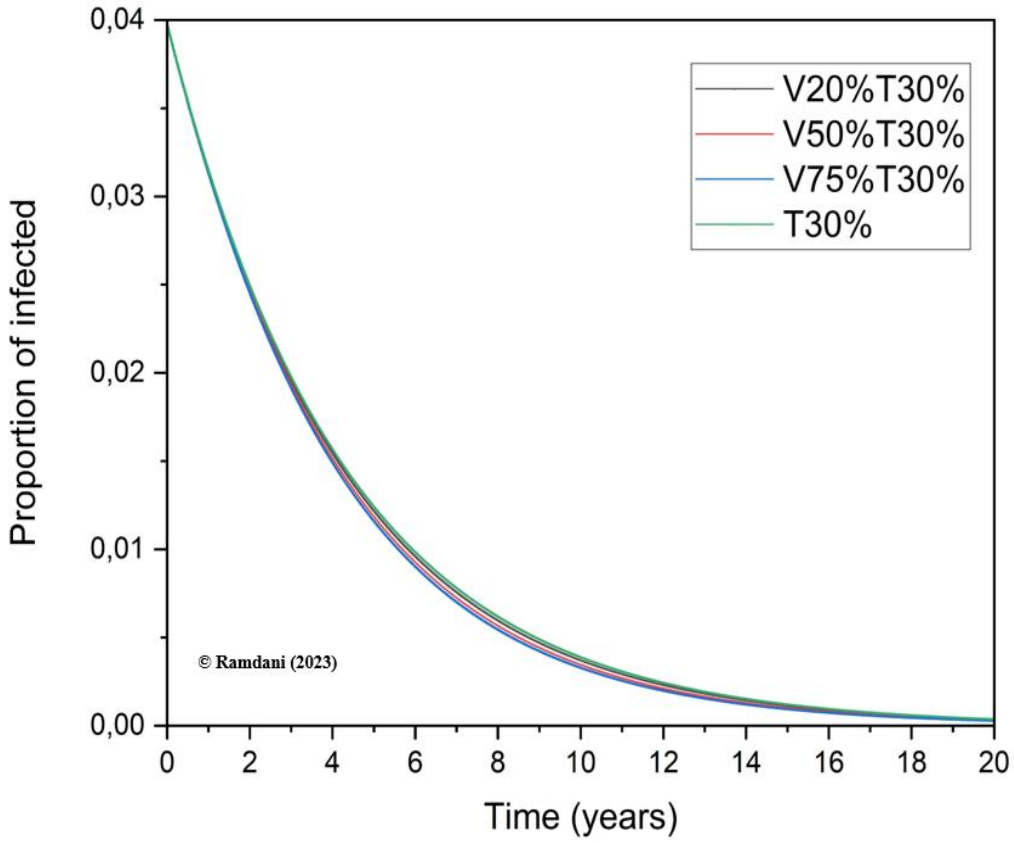

Figure 26: Simulation of dynamic of brucellosis in small ruminant after 30% of test-and-slaughter (T) alone and combined with vaccination (V) for 20 years

The figure 26 represents a line graph that manifests the proportion of infected animals after implementing interventions based on test-and-slaughter of 30% of adults alone and combined with vaccination of 20%, 50% and 75% of young animals over the upcoming 20 years.

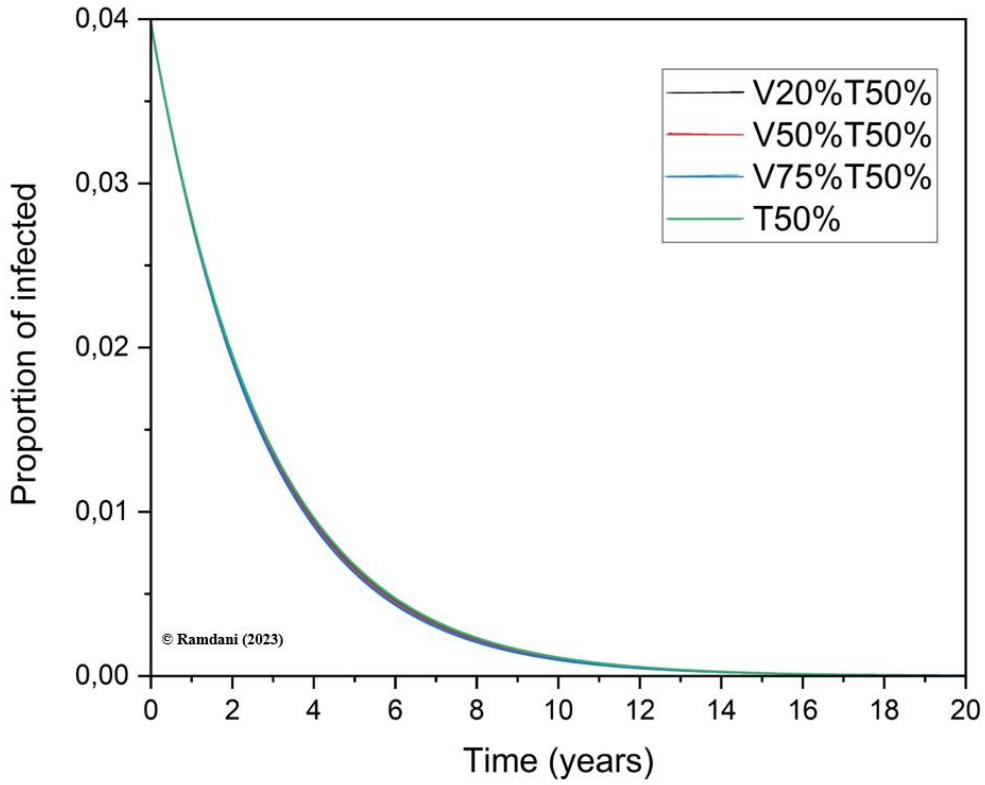

Figure 27: Simulation of dynamic of brucellosis in small ruminant after 50% of test-and-slaughter(T) alone and combined with vaccination (V) for 20 years

The figure 27 represents a line graph that reports the proportion of infected animals after implementing strategies based on test-and-slaughter of 50% of adults alone and combined with vaccination of 20%, 50% and 75% of young animals over the upcoming 20 years.

Prevalence rates of brucellosis after simulation of test-and-slaughter of 5%, 10%, 15%, 20%, 30% 50% and 75% of animals over 20 years revealed to be: 0.80%, 0.44%, 0.24%, 0.13%, 0.04%, 0% and 0%, respectively. Further results of the simulation of this control strategy are illustrated in figures 21, 25, 26, 27 and 28 and appendix 6.

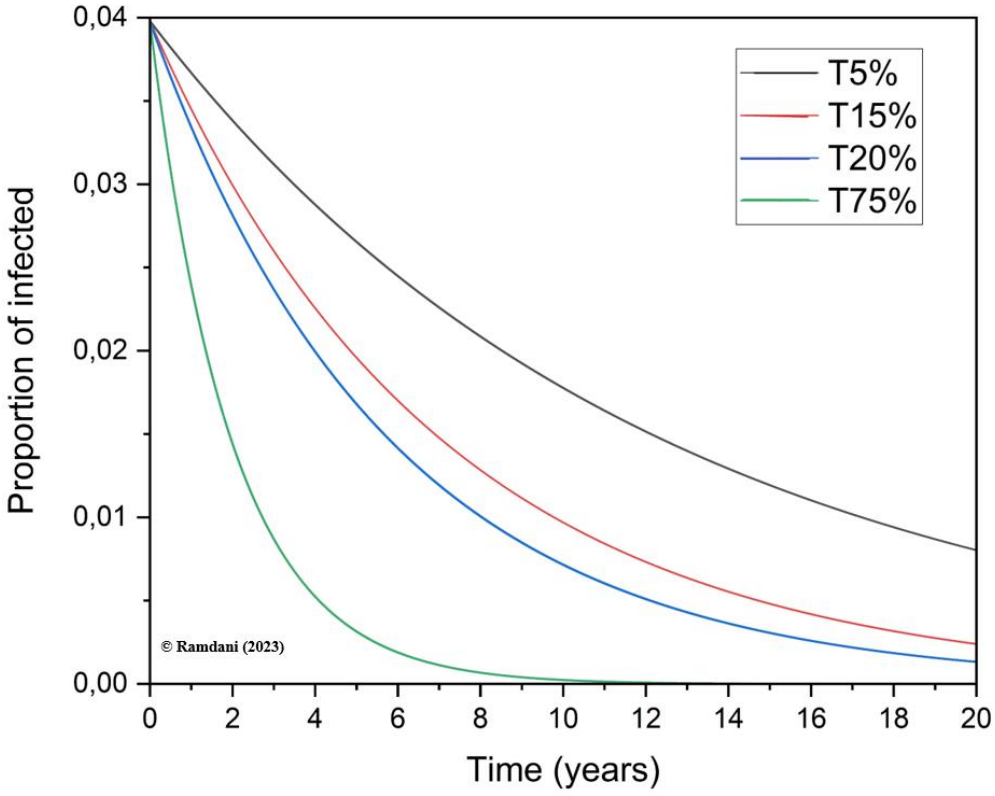

Figure 28: Simulation of dynamic of brucellosis in small ruminant after testing-and-slaughter (T) of 5%, 15%, 20% and 75% of adults for 20 years

The figure 28 represents a line graph that illustrates the proportion of infected animals after application of policies based on test-and-slaughter of 5%, 15%, 20% and 75% of adults over the upcoming 20 years.

Figure 21 demonstrates a bar chart of simulated prevalence of brucellosis expected after 5, 10 and 20 years in each mathematical model. Overall, the bar graph shows a sharp drop of infected proportion after 5, 10 and 20 years of application of policies based on test and slaughter strategy, specifically over 30 % of adults. Indeed, a significant decrease in prevalence can be observed after 5 years of vaccination of 20%, 50% and 75% of young animals combined with testing and elimination of 50% of adults to register prevalence estimates of: 0.66%, 0. 64% and 0.63%, respectively. Similarly, after 5 years of application of test and slaughter of 50% and 75% of adults, infected rates have shrunk notably to record 0.67% and 0.31%, respectively.

After 10 years, the same strategies revealed similar decrease in prevalence of brucellosis. Furthermore, policies related to 30 % of test and slaughter of adults showed a remarkable drop of infected individual rates. A notable retreat of infected animals can be seen through the bar chart after 20 years of adopting control measures based on 10% of test-and-slaughter combined with vaccination, 15% and 20% of only test-and-slaughter of adults.

Additionally, vaccination of 90% of young sheep and goats resulted in prevalence less than 0.06 % altogether with interventions based on 30 % of test-slaughter after 20 years of application. At the final phase of the simulation, policies related to test and slaughter of positive reactors over 30 % succeeded to eradicate the disease.

The bar graph in figure 29 manifests the percentage of vaccination coverage simulated at 5, 10 and 20 years for the three vaccination strategies (mass vaccination, vaccination of young and adult vaccination). In general review, the vaccination of young animals resulted in the highest percentage of vaccination coverage (>60%) at 75%, 90% and 100% vaccination proportion for more than 10 years of application. However, mass vaccination gave the highest percentages of vaccination coverage at the lowest vaccination rates (25%: 31.83% to 33.29%) and (50%: 48.52% to 49.86%), followed by the adult vaccination (25%: 22.63% to 23.77%) and (50%: 32.61% to 33.81%).

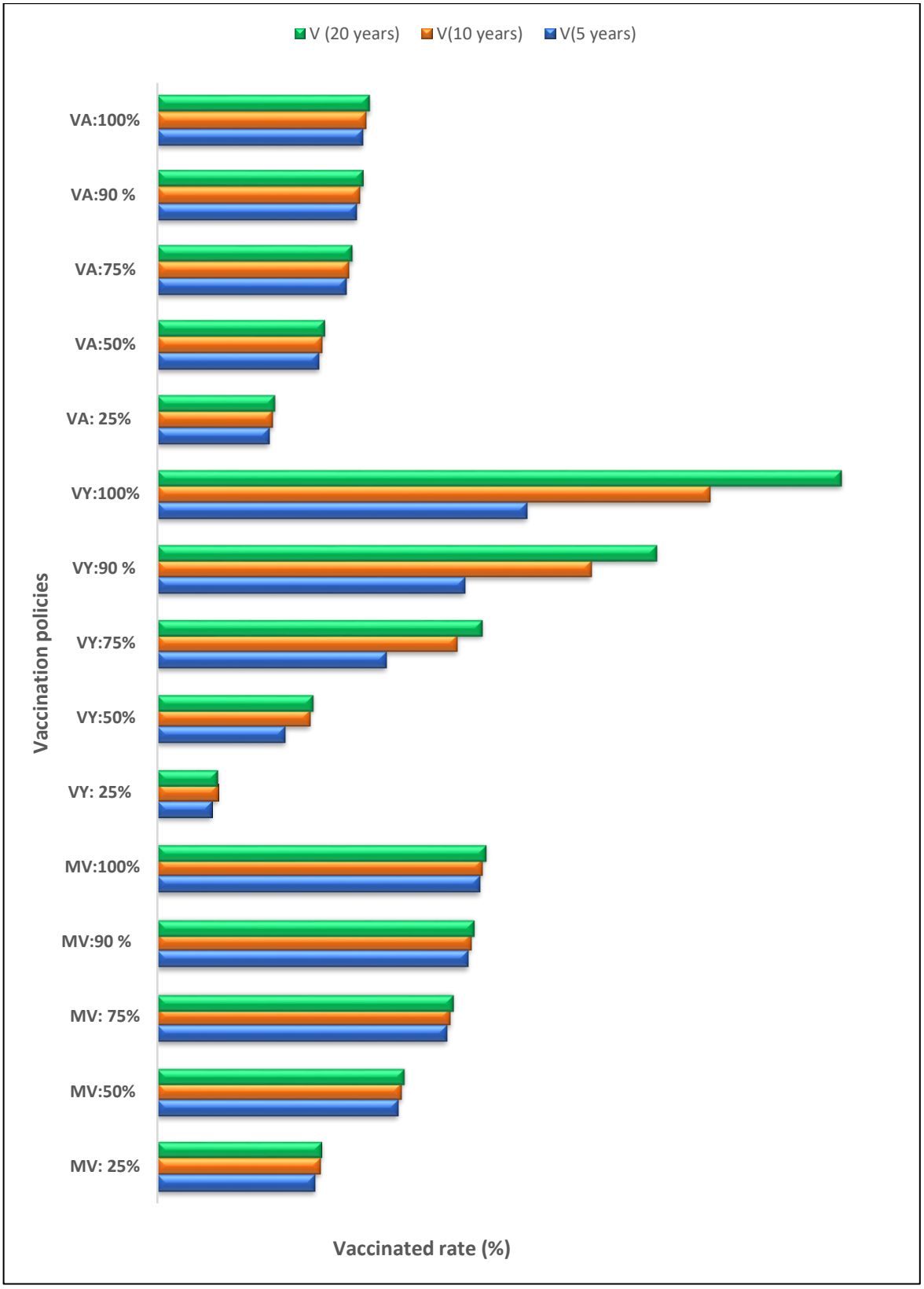

Figure 29: Rate of vaccinated (VA: adults, VY: young and MV: mass vaccination) in vaccination policies in 5, 10 and 20 years

The figure 29 represents a bar graph that showcases the vaccinated rate in the population of small ruminant in El Oued area after 5, 10 and 20 years of implementing strategies based on mass vaccination, vaccination of young animals and vaccination of adults at proportions of 25%, 50%, 75%, 90% and 100%.

4. Discussion

Our study provided a simulation of the outcome of implementation of various control strategies of small ruminant brucellosis that might have been of immeasurable worth without warranted results if applied in the field. The simulated policies were derived from literature (Benkirane, 2006; Minas, 2006). Proportions of vaccination and sampling of animals to be slaughtered after test confirmation were selected randomly. However, the feasibility of application was taken into consideration. The mathematical models were developed to be as realistic as possible. Indeed, data related to *Brucella melitensis*, characteristics of animal population in the study area, Rev 1 vaccine efficiency, post-vaccination immunity, history of vaccination and the performance of serological tests that are low-cost and validated by the OIE, were collected with scrupulous consideration.

Forecasting the dynamic of brucellosis transmission in small ruminants showed that the prevalence is decreasing gradually and slowly. After decades, brucellosis can be eradicated totally without any intervention. However, other factors can intervene in the course of the transmission dynamic such as animal movement between provinces and other neighboring countries such as Tunisia.

Taking the prevalence as an indicator of the effectiveness of a control policy. Our findings revealed that test and slaughter is the most effective strategy in order to eradicate the disease. Sampling 50% or more of adult animals to be slaughtered after positive reaction either combined with vaccination or alone can result in an expeditious decline in prevalence. This strategy might be adopted for controlling the disease for about 5 or10 years to bring down the prevalence below 1% and 0.11%, respectively. It also succeeded to eradicate the disease in 13 years if 75% of adults would be sampled to be eliminated. Whereas, sampling 50% of adults solely or combined with vaccination led to eradication of brucellosis in 18-19 years.

It has to be brought to mind that proportion of sampling can be largely higher than the percentage of elimination of seropositive animals. For instance, sampling 50 % of adults would result in elimination of 0.85%, 0.04% and 0.01% of the total population after one year, 10 years and 20 years of execution of the policy.

Combining vaccination of young animals to test-and-slaughter policy can accelerate slightly the downturn of the disease. For instance, vaccination of 20 % to 75 % of young animals besides test-and-slaughter of 50% of adults reduced the infected rate by 0.01 % to 0.04 %. Therefore, adopting vaccination in conjunction with test-and-slaughter is not a cost-effective scheme. Nevertheless, the effectiveness of vaccination can be slightly improved when sampling animals for elimination at lower rates (10%) during 10 years. Indeed, vaccination of 75 % in conjunction with 10% test-and-slaughter can diminish the prevalence to 1.11% in comparison to 1.31% of only test-and-slaughter approach.

The impact of vaccination strategies on the prevalence of the disease is insignificant in comparison to other policies. At lower percentages of vaccination proportion (25%, 50% and 75%); the mass vaccination was proven to be the most efficient vaccination approach. However, at higher vaccination proportions (90% and 100%) and for long term of execution (20 years), vaccination of young animals should be considered a priority among vaccination strategies to lower the prevalence to less than 1%. Nevertheless, the difference is generally marginal between all vaccination policies at various proportions, particularly for a short and medium period of time (5-10 years). The largest gap in prevalence was 0.33% recorded between vaccination of 25 % of young animals and vaccination of the entire population (100%) for 10 years.

Our results are in line with the recommendations of Minas (2006) and Smits (2013). Minas (2006) suggested the adoption of a program combining vaccination of young animals and test-and-slaughter approach in case the individual prevalence was between 1% and 5 %. Indeed, this policy was proven to be the most effective in our simulation starting by the prevalence estimate of 3.98%. However, our findings contradict partially the conclusions of Minas (2006). Combining vaccination to test-and-slaughter policy was found to be of minor effect in comparison to test-and-slaughter only. Benkirane (2006) and Blasco (2010) described the same point of view as Minas (2006), with the exception of herd prevalence in the place of prevalence of animals, which did not match our data.

In comparison to similar mathematical models, our results match the findings of Hegazy et al. (2021) in Egypt. They proved the effectiveness of test-and-slaughter policy alone and combined with vaccination of young animals in low seroprevalence areas. Additionally, they concluded on mass vaccination being the most promising among various vaccination approaches.

Contrary to our results, combining vaccination of adults and young replacements at different percentages reduced significantly their initial seroprevalence. Moreover, as a result of their simulation modeling of brucellosis, a considerable difference was found between vaccination of young animals and adults. This disagreement in outcomes might be explained by the high level of their initial seroprevalence (15.5%) in comparison to ours (3.98%). Indeed, as recited by previous authors (Benkirane, 2006; Minas, 2006; Blasco, 2010), at high initial seroprevalence (>5%), mass vaccination would be the first procedure in control programs to bring down the prevalence to an acceptable level.

Ten (10) years ago, Aïnseba et al. (2010) simulated the dynamic of transmission of ovine brucellosis in Algeria over 10 years. In contrast to our results, they found that the disease would increase over the first five years from then to reach a plateau of stability around 65 %. They also reported the ability of slaughtering policy without testing to eradicate the disease during 10 years. This controversy is probably due to overestimation of the infectiousness of newborns of infected females in their model. Whereas, elimination of animals regardless of their health status could not be considered a practical procedure to reduce and eradicate the disease.

Cross-species transmission of *Brucella* should be taken into consideration when modeling and simulating brucellosis in other areas of Algeria. In that case, collection precise and reliable data would be challenging. Cattle and camel role in the dynamic of transmission of brucellosis were neglected in our study for a number of reasons. Firstly, lack of data regarding true prevalence of brucellosis. The second reason was that our study area possessed about 93% of small ruminants among livestock.

5. Conclusion

Based on our findings, we conclude that sampling of 50% of adults to be eliminated after testing positive by series of serological tests (RBT and CFT) for 20 years would be considered the optimum strategy to eradicate the brucellosis. Combining vaccination of young animals would not be cost-effective for long-term program. Simulating the course of dynamic of transmission of brucellosis on a larger level and including other species is highly recommended to have optimum results in other parts of Algeria.

General Discussion

Brucellosis is an endemic zoonotic disease in Algeria, responsible for thousands of human infections every year. Multiple attempts of the disease control were implemented in different species since 1970 (MADR, 2021). The decision makers in Algeria focused more on controlling bovine brucellosis at the beginning. Afterward, goats were included, followed by sheep after a decade. This mindset came from the fact that dairy products consumption constituted the main route of transmission. Moreover, this program was addressed more to northern provinces in Algeria where cattle represented a considerable proportion of livestock. However, control policies should be detailed to each ecologically distinct area discerned by epidemiological, geographic and socio-economic conditions in addition to characteristics of animal population and farming systems.

From these points, our main objective was to design a control/eradication strategy in El Oued district. The latter is a bordering province near the Tunisian border and characterized by its dry climate and desertic and oasis geography. DSP of El Oued reports hundreds of humans cases every year (DSP El Oued, 2021). According to the epidemiological investigation of the veterinary authority, most cases are caused by caprine brucellosis. Sheep and goats in this region represent 93% of livestock (DSA El Oued, 2021). Most goats are mixed with sheep in livestock premises. Consequently, we targeted small ruminant brucellosis in this area to create a control/eradication policy adjusted to the local data. Firstly, we carried out a cross sectional study in order to determine the true prevalence and study the epidemiology of the disease in small ruminant flocks. The sampling was proportional to the size of sheep and goats in each municipality. Therefore, all municipalities were included in the study to illustrate the distribution of the disease in El Oued. The relative results showed a true herd prevalence of 27.95% and individual true prevalence of 3.98%. Northern and eastern municipalities as well as Chief town municipality recorded the highest prevalence estimates, followed by municipalities located in the southernmost points of the province with intermediate rates of brucellosis. However, western areas showed zero brucellosis seropositivity. History of abortion revealed to increase five (5) times the odds of seropositivity of brucellosis in herds of sheep and goats. Presence of chicken and pigeons within flocks showed to lower the likelihood of the disease. We explained this protective role of poultry by serving as insect control due to the possibility of vector transmission of brucellosis.

Secondly, we aimed to develop a deterministic mathematical model of the dynamic of transmission of the disease. For that, we collected and searched indispensable data that was related to *Brucella melitensis* pathogenesis and epidemiology, and characteristics of animal population in the study area on top of the true individual prevalence resulted from the first study as a starting point.

Simulating the course of the disease over 20 years resulted in a gradual decrease of the prevalence from 3.98% to 1.47%. Thirdly, we have developed a deterministic simulation agent-based-models that have included three policies of control and eradication of brucellosis with a set of varied parameters such as proportion of sampling for testing and vaccination rate. Policies included approaches of vaccination, combination of vaccination and test-and-slaughter, and test-and-slaughter only. For that, data related to Rev 1 vaccine efficiency, waning immunity, history of vaccination and the performance of serological tests validated by the OIE was gathered to be included in the simulation models. The results showed that the best method for eradicating the disease is test- and-slaughter strategy. A rapid drop in prevalence below 1% and complete eradication of brucellosis could be achieved by sampling 50% or more of adult animals that will be slaughtered after a positive reaction, either in conjunction with vaccination or alone. Combining test-and-slaughter practices with immunization of young animals could boost the disease decline a little faster. The latter, however, is a less economical plan. For all vaccination strategies at different proportions, the difference was typically negligible, especially for short- and medium-term effects (5-10 years).

Our study manifested the significance of mathematical modeling in studying and predicting the dynamic of animal infectious diseases in Algeria, in addition to simulation of control strategies and adopting the most effective and economical strategy. Nevertheless, for the mathematical model of the dynamic of transmission of brucellosis to be as realistic as possible, it should include all sensitive species including humans. Moreover, essential number of parameters related to the epidemiology and pathogenesis of brucellosis should be taking into consideration: 1) The seasonality of contagion and transmission of the disease, particularly, during parturition period of time. 2) Age meaning the absence of transmission of *Brucella* among young animals .3) Possible transmission in-utero from infected mother to offspring.4) Movement of animals to and out of the study area. However, some data is challenging to obtain. Additionally, including all the aforementioned elements would make the model more complex, hence, difficult to develop.

Our thesis came to the conclusion that adopting a test-and-slaughter policy would be the most cost-effective strategy to control or eradicate the brucellosis in small ruminant for a long-term program. However, complementary measures should accompany this approach such as biosecurity measures and insect control within the herd premises. However, our findings apply to the study area, which leads to our recommendation for conducting similar studies in other parts of Algeria using the regional required data for specific results.

General conclusion and recommendations

General conclusion

Pertinent and updated literature review on brucellosis and *Brucella* sp. and modeling of infectious diseases was detailed in the present dissertation.

Our PhD project provided relevant results regarding the epidemiological situation of small ruminant brucellosis in El Oued province. The cross-sectional study revealed the true herd prevalence without any bias by taking into consideration the imperfection of the used serological tests and the history of vaccination. The distribution of infection of *Brucella* spp. across the study area was also demonstrated. The study of risk factors within the herds showed the association of abortion and poultry presence to brucellosis seropositivity. Modeling of the dynamic of brucellosis in small ruminant revealed the possibility of reducing the prevalence of the infection without any intervention. Whereas, the simulation of different control strategies over 20 years demonstrated that sampling of 50% of adults and elimination of positive reactors to both tests RBT and CFT used in series would be the optimum policy to eradicate the disease. Additional measures related to biosecurity at farms and insect control should be implemented in conjunction with the control/eradication policy for an effective and successful control scheme.

Recommendations

Thorough epidemiological studies should be carried out within ecologically distinct areas for the control strategy to be detailed to each distinguished region.

Moreover, different models of the spread of brucellosis should be developed depending on the epidemiological conditions in each area.

Furthermore, application of mathematical modeling and epidemiological simulation to evaluate control strategies of animal diseases is highly recommended in Algeria to design a cost-effective policy.

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Appendices

Appendix 1: Number of small ruminant herds to be sampled per municipality

Number	Municipality	Small Ruminant Number	Percentage (%)	Number of herds to be sampled	Rounding of figures
01	El Oued	37 400	3,73	1,83	2
02	Robbah	50 800	5,06	2,48	2
03	Oued –Alanda	17 000	1,69	0,83	1
04	Bayada	15 200	1,52	0,74	1
05	Nakhla	51 800	5,16	2,53	3
06	Guemar	28 000	2,79	1,37	1
07	Kouinine	7 400	0,74	0,36	1
08	Reguiba	63 800	6,36	3,12	3
09	Hamraia	8 800	0,88	0,43	1
10	Taghzout	4 650	0,46	0,23	1
11	Debila	24 500	2,44	1,20	1
12	Hassani Abdelkrim	26 500	2,64	1,29	1
13	Hassi Khalifa	122 400	12,20	5,98	6
14	Taleb Larbi	89 900	8,96	4,39	4
15	Douar El-Maa	98 200	9,79	4,80	5
16	Sidi Aoun	28 500	2,84	1,39	1
17	Trifaoui	35 600	3,55	1,74	2
18	Magrene	61 000	6,08	2,98	3
19	Ben Guecha	148 000	14,75	7,23	7
20	Ouermes	5 100	0,51	0,25	1
21	Ogla	43 900	4,38	2,14	2
22	Mih Ouensa	34 600	3,45	1,69	2
Total		1 003 050	100	49	51

Appendix 2: Questionnaire administrated to animal owners

1). Characteristics of the herd and the owner	
Flock number	
Owner name	
Date	
1. Adress	-Region: -Municipality: -Province:
2. Herd owner work time	-Fulltime -Part-time
3. Production system	-Intensive -Semi-extensive -Agropastoral
4. Herd composition	-Unified -Mixed
5. Production type	-Commercial -Traditional
6. Herd size	-<100 -≥100
7. Species in the farm	-Goats – Sheep – Cattle – Camel
8. Other species in the farm	
9. Number of sampled animals	-Sheep -Goat
2) History of health issues	
10. Abortion (in the last 12 months)	-Yes -No
11. Retained placenta (in the last 12 months)	-Yes -No
12. Stillbirth (in the last 12 months)	-Yes -No
3). Herd management	
13. Abortion management	-Burn -Burying -Public wasteland -Giving to dog
14. Contact with wild animals	-Yes -No
15. Contact with other herds	-Yes -No
16. Isolation of parturient	-Yes -No
17. Isolation of unhealthy animal	-Yes -No
18. Renewing of herd (both: self-reproduction and purchase)	Self-reproduction Purchase Both
19. Quarantine of newly introduced animals	-Yes -No
20. Cleaning frequency	-Rarely -Never -Frequently
21. Frequent visitors	-Yes -No
22. Reproducer male (outsider: borrowed or purchased)	-Outsider -Raised

Appendix 3: Rose Bengal test procedure

The test was conducted according to the fabricant's instructions (Lillidale Diagnostics ®, Dorset, United Kingdom) as follows:

- 30 ul of each serum sample was placed on the agglutination plate.
- 30 ul of the antigen was placed beside the serum sample on the agglutination plate.
- The antigen and the serum were mixed with a stirring rod.
- The plate was shacked for 4 minutes using a plate shaker.
- Results interpretation:
    - No agglutination indicated negative sample
    - Agglutination indicated positive sample

Appendix 4: Indirect ELISA procedure

- The wells (12X8 in each plate) were coated with LPS from *B. abortus*
- The samples to be tested and the controls were distributed in the wells, diluted to 1/20. Anti-*Brucella* antibodies, if present, form an antigen-antibody complex.
- A multi-species conjugate labeled with peroxidase (HRP) was distributed in the wells. It bound to anti-*Brucella* antibodies, forming an antigen-antibody-conjugate-HRP complex.
- After removal of excess conjugate by washing. The reaction was revealed by a revealing solution (TMB).
-The resulting coloration was related to the number of specific antibodies present in the sample to be tested.

- In the presence of antibodies in the sample, a blue color appeared which turned yellow after blocking.
- In the absence of antibodies in the sample, no color appeared.

-The reading of microplates was carried out at a wavelength of 450nm using ELISA Microplate Reader.

-Each plate test was valid when:

- The mean value of optical density of positive controls sera (DOcp) was superior than 0.350: DOcp > 0.350
- The ratio of the mean of the positive controls (DOcp) and the mean of the negative controls (DOcn) is greater than 3: DOcp/DOcn>3.

-Interpretation:

- For each sample, the percentage of positivity S/P% was calculated as follows:

$$\frac{S}{P}\% = \frac{DOsample - DOcn}{DOcp - DOcn} \times 100$$

- If S/P% ≤110%, the sample was considered negative
- If 110% < S/P% <120%, the sample was considered doubtful
- If S/P% ≥120%, the sample was considered positive

-The doubtful samples were retested to obtain a binary result (negative or positive).

-The final samples that showed doubtful results were considered positive.

Appendix 5: Complement fixation test procedure

1. Reagents and diluents

- Antigen: Suspension of *B. abortus* biovar 1 (Weybridge 99 strain), inactivated by heat and phenol. It is calibrated to give 50% hemolysis with the French sub-standard of the international standard (OIEISS) diluted to 1/200.
- Complement Fixation Test Buffer (CFTB): is a concentrated buffer (20X) pH 7.2, used to dilute sera and reagents in complement fixation reactions. It maintains precise pH conditions, and reduces the number of anti-complementary serums. The presence of Ca^{++} and Mg^{++} ions enhances the activity of complement.
- Guinea Pig Complement.
- Haemolytic system: consisted of a mixture of equal parts of:

  - Suspension of red blood cells at 2.5%.

  -Dilution of rabbit haemolytic serum anti-sheep-red-cells containing two haemolytic units 100%.

2. Preparation of reagents and diluents

2.1. Preparation of the Complement Fixation Test Buffer (CFTB): Dilution the CFTB to 1/20 in distilled water.

2.2. Preparation of suspension of red blood cells:

- Preparation of ALSEVER's solution:

The Alsever's solution is a saline liquid used as an anticoagulant. It was prepared using the following components:

-20 g of Glucose

-8 g of Sodium citrate

-4.2 of Sodium Chloride

- 0.365 g of Citric acid

-100 ml of distilled water

- Preparation of sheep red blood cells:

- Blood was drawing from a sheep in tubes containing Alsever's solution.
- Red blood cells were centrifuged and washed three times. Afterwards, they were stored in the Alsever's solution at the refrigeration temperature.

2.3. Preparation of haemolytic serum:

- The haemolytic serum was diluted to 1/1000 in CFTB.

2.4. Preparation of haemolytic system:

- The 2.5 % suspension in CFTB of red cells was prepared and mixed with the haemolytic serum at an equal volume.

2.5. Antigen preparation:

- The antigen was diluted to 1/200 in CFTB

2.6. Complement titration:

-After titration of complement, dilution of 120 ul of complement gave haemolytic unit 50% (H50).

- Therefore, the volume of complement (Cv) to be used for the test of our samples was calculated based on the following formulae:

$$\text{Cv (ul)} = \frac{120 \times 6 \times \text{N} \times 25}{100 \times 200}$$

- 120: the value of complement found in complement titration
- 6: six units of complements in H50
- N: number of tests (samples and controls) to be conducted
- 25: Volume of complement in the test
- 100: the dilution of the complement in titration
- 200: the volume of antigen in titration
  - Cv was diluted in ((25 x N)- Cv) ul.

3. The procedure: the method used was cold complement fixation in microplates (U bottom).

-Heat inactivation of sera for 30 min in a water bath at 60°C ± 2°C

-Dilution of sera: sera were diluted to ¼ in CFTB (25 ul of each inactivated serum was mixed with 75 ul of CFTB) and were put in a microplate of diluted sera.

-The diluted serum, the antigen, the complement, the haemolytic system and the CFTB were distributed in the wells of the test microplate.

-Controls of the antigen, the complement and the haemolytic system were distributed as follows:

	CFTB	Antigen	Complement
Antigen control	25 ul	25 ul	25 ul
Complement control	50 ul	/	25 ul
Haemolytic system control	75 ul	/	/

-Reading and interpretation of results:

- Reading of the microplate was carried out with the use of a mirror.
- The test was validated by the results of controls that are supposed to be:
  - ✓ Antigen control: 100% hemolysis.
  - ✓ Complement control: 100% hemolysis.
  - ✓ Hemolytic system control: 0% hemolysis.
- Sera that showed anti-complementary activity were inactivated a second time and retested.
- 50% hemolysis of diluted serum to ¼ is equivalent to: antibodies titer of 20 UI/ml.
- Serum that showed more than 20 UI/ml was considered positive (OIE, 2018)
- Thus, Serum that showed hemolysis ≤50% hemolysis was considered positive.
- 50% Hemolysis control was obtained from the complement titration.

Appendix 6: Proportions of infected (I), susceptible (S) and vaccinated (V) at 5, 10 and 20 years of simulated mathematical models

Policy number	Strategy	5 years	10 years	20 years
Model null	Zero intervention	I:0.0309	I:0.0240	I:0.0147
#1	MV: 25%	I:0.0291 S:0.6526 V:0.3183	I:0.0209 S:0.65 V:0.3289	I:0.0109 S:0.6568 V:0.3329
#2	MV:50%	I:0.0285 S:0.4863 V:0.4852	I:0.0201 S:0.4868 V:4931	I:0.0100 S:0.4921 V:0.4986
#3	MV:75%	I: 0.0282 S: 0.3872 V:0.5847	I:0.0197 S:0.3891 V:0.5913	I: 0.0096 S: 0.3935 V:0.5977
#4	MV:90 %	I:0.0280 S:0.3451 V:0.6269	I:0.0195 S: 0.3472 V:0.6332	I:0.0095 S:0.3513 V:0.6401
#5	MV:100%	I:0.02795 S:0.3219 V: 0.6502	I:0.0194 S:0.3240 V:0.6565	I:0.0094 S:0.3269 V:0.6636
#6	VY: 25%	I:0.0302 S:0.8599 V: 0.1099	I:0.0227 S: 0.8543 V: 0.1230	I:0.0128 S:0.8649 V:0.1227
#7	VY:50%	I:0.0296 S:0.7123 V: 0.2582	I: 0.0214 S:0.6704 V:0.3081	I:0.0112 S:0.6745 V: 0.3148
#8	VY:75%	I:0.0289 S:0.5093 V:0.4617	I:0.0204 S:0.3744 V: 0.6052	I: 0.0099 S:0.3350 V: 0.6559
#9	VY:90 %	I: 0.0287 S: 0.3509 V: 0.6203	I: 0.0198 S:0.1045 V:0.8757	I: 0.0005 S: -0.0069 V: 1.0077

Appendix 6: Proportions of infected (I), Susceptible (S) and Vaccinated (V) at 5, 10 and 20 years of simulated mathematical models (Continued)

#10	VY:100%	I:0.0285 S:0.2259 V:0.7456	I:0.0195 S: -0.1347 V:1.1152	I: 0.0089 S: -0.3882 V: 1.3809
#11	VA: 25%	I: 0.0295 S: 0.7442 V: 0.2263	I:0.0216 S: 0.7452 V: 0.2332	I: 0.0116 S: 0.7512 V: 0.2377
#12	VA:50%	I:0.0289 S: 0.6449 V: 0.3261	I: 0.0209 S: 0.6472 V: 0.3319	I: 0.0109 S: 0.6516 V: 0.3381
#13	VA:75%	I: 0.0287 S: 0.5908 V: 0.3805	I: 0.0205 S: 0.5930 V: 0.3865	I:0.0105 S: 0.5966 V:0.3935
#14	VA:90 %	I:0.0286 S: 0.5688 V: 0.4026	I: 0.0204 S: 0.5708 V: 0.4088	I: 0.0104 S: 0.5741 V: 0.4162
#15	VA:100%	I: 0.0285 S: 0.5568 V: 0.4147	I: 0.0203 S:0.5587 V: 0.4209	I: 0.0103 S: 0.5618 V: 0.4286
#16	VTS: V:20%/TS:10%	I:0.0224 S: 0.8932 V: 0.0844	I: 0.0125 S: 0.8946 V: 0.0929	I: 0.0039 S: 0.9041 V: 0.0924
#17	VTS: V:20%/TS:30%	I: 0.0122 S: 0.9047 V:0.0832	I: 0.0037 S:0.9049 V: 0.0913	I: 0.0003 S: 0.9084 V: 0.0916
#18	VTS: V:20%/TS:50%	I: 0.0066 S: 0.9110 V: 0.0823	I: 0.0011 S:0.9082 V: 0.0907	I:2.9888e-05 S:0.9089 V: 0.0915
#19	VTS: V:50%/TS:10%	I: 0.0218 S: 0.7225 V: 0.2557	I: 0.0117 S: 0.6849 V: 0.3034	I:0.0033 S: 0.6873 V: 0.3099
#20	VTS: V:50%/TS:30%	I: 0.0119 S:0.7361 V: 0.2521	I:0.0035 S: 0.6981 V: 0.2985	I: 0.0003 S: 0.6929 V: 0.3074
#21	VTS: V:50%/TS:50%	I:0.0064 S:0.7439 V: 0.2497	I: 0.0010 S: 0.7026 V: 0.2964	I: 2.5311e-05 S: 0.6935 V: 0.3069

Appendix 6: Proportions of infected (I), susceptible (S) and vaccinated (V) at 5, 10 and 20 years of simulated mathematical models (Continued)

#22	VTS: V:75%/TS:10%	I: 0.0214 S:0.5211 V: 0.4575	I: 0.0111 S: 0.3923 V: 0.5966	I: 0.0029 S: 0.3515 V: 0.6465
#23	VTS: V:75%/TS:30%	I: 0.0116 S: 0.5371 V: 0.4513	I: 0.0033 S: 0.4093 V: 0.5874	I: 0.0003 S: 0.3595 V: 0.6411
#24	VTS: V:75%/TS:50%	I:0.0063 S:0.5465 V: 0.4472	I: 0.0009 S: 0.4158 V: 0.5832	I: 2.22e-05 S:0.3609 V: 0.6400
#25	TS: 5%	I: 0.0265 S: 0.9735	I:0.0178 S: 0.9822	I: 0.0080 S: 0.9923
#26	TS: 10%	I: 0.0228 S:0.9772	I:0.0131 S: 0.9869	I: 0.0044 S: 0.9959
#27	TS: 15%	I: 0.0196 S: 0.9804	I: 0.0097 S: 0.9903	I: 0.0024 S: 0.9979
#28	TS: 20%	I:0.0169 S: 0.9832	I: 0.0072 S: 0.9929	I:0.0013 S: 0.9989
#29	TS: 30%	I: 0.0124 S: 0.9876	I:0.0039 S: 0.9961	I: 0.0004 S: 0.9999
#30	TS: 50%	I:0.0067 S: 0.9933	I: 0.0012 S: 0.9989	I: 3.3505e-05 S: 1.0002
#31	TS: 75%	I: 0.0031 S: 0.9969	I:0.0003 S: 0.9998	I: 1.5785e-06 S: 1.0003

Appendix 7: Published article entitled: "Brucellosis in small ruminant: seroprevalence, risk factors, and distribution in the southeast of Algeria" in Tropical Animal Health and Production Journal.

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REGULAR ARTICLES

Brucellosis in small ruminant: seroprevalence, risk factors, and distribution in the southeast of Algeria

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Abstract

The impact of brucellosis on public health and economy is unquestionable in developing countries such as the case of Algeria. This study aimed to provide further understanding of epidemiological status of brucellosis in small ruminant flocks in the southeast of Algeria. Therefore, a cross-sectional study was conducted among small ruminant flocks ($n=51$) in El Oued district using simple random sampling strategy. The serum samples collected from 612 sheep and goats (sheep = 280, goats = 332) were screened for *Brucella* antibodies using the Rose Bengal test (RBT) and the indirect enzyme-linked immunosorbent assay (iELISA) in parallel on all the serum samples. The seropositive serum samples of both tests were confirmed with the complement fixation test (CFT). A structured questionnaire regarding animal, herd, and farm management was prepared and completed in parallel to sampling. Association between variables and *Brucella* spp. seropositivity status of herds was assessed by univariate and multivariate analysis using simple and binary logistic regression. Estimated true herd prevalence was 27.95% (95% CI, 17.18–42.01), and true individual prevalence was 3.98% (95% CI, 2.51–6.03). Seropositive herds were detected in bordering areas and regions with the highest livestock density. Occurrence of abortions in herds ($p=0.03$) increased at least five times (5) the odds of being seropositive (OR, 6.25; 95% CI, 1.20–32.46). Poultry presence in farms revealed to be a protective factor ($p=0.01$) (OR, 0.11; 95% CI, 0.02–0.61). The high-level seroprevalence quantified in this study in small ruminant flocks reflects the persistent animal infection endemicity and the high risk of human exposure.

Keywords Algeria · Brucellosis · Goats · Risk factors · Seroprevalence · Sheep

Introduction

Brucellosis is a bacterial anthropozoonotic, caused by several species of the genus *Brucella*. Six classical species identified by their antigen/biochemical characteristics and primary host species are responsible for the animal disease: *B. abortus* (cattle), *B. melitensis* (sheep and goats), *B. suis* (pigs), *B. ovis* (sheep), *B. canis* (dogs) and *B. neotomae* (rodents) (Ficht 2010). However, cross-species transmission of *Brucella* spp. has been detected (Wareth et al. 2015; Alamian and Dadar 2020; Lounes et al. 2021). Only four species are pathogenic for humans (*B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*). *B. melitensis* is the most invasive and virulent species for humans (Whatmore et al. 2016). Brucellosis ranks first in the list of zoonotic bacterial diseases, and 500,000 cases are reported annually in endemic areas (Pappas et al. 2006). For the study area alone, hundreds of human cases of brucellosis have been reported each year according to the Directorate of Health and Population of El Oued (DSP El Oued 2020).

Human brucellosis is classified as an acute, sub-acute, or chronic febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia, and arthralgia (Doganay and Aygen 2003; Corbel 2006). Human brucellosis is acquired mainly through consumption of contaminated raw milk and unpasteurized dairy products, contact with infected animals, and inhalation of contaminated aerosols (OIE 2018).

Animal brucellosis is a chronic disease characterized mainly by reproductive disorder. *Brucella* affect mainly the reproductive tract of animals, causing low fertility rate, abortion, placenta

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retention, and stillbirths in females while causing orchitis, epididymitis, and uni- or bilateral testicular atrophy, sperm abnormalities, and infertility in males (Megid et al. 2010). In addition to its impact on human health, the economic impact of brucellosis on the livestock population is described mostly by reproductive losses as the increase in abortion and stillbirth, a decrease in fertility, a decline in milk production, and a longer calving interval (Akakpo et al. 2010; Franc et al. 2018).

Brucellosis is widespread worldwide. Algeria is reported to be one of the countries with the highest incidence according to the World Organisation for Animal Health (OIE 2021). Since 1970, several control strategies have been established against bovine brucellosis in Algeria. During 1995–2006, goats were included into test–slaughter program all together with cattle. However, since 2006 until 2017, vaccination of young small ruminants (sheep and goats) with Rev-1 was integrated into the previous strategy (MADR 2021). Despite all these efforts, the incidence of brucellosis showed a fluctuation with epidemic peaks during the last decade.

Benkirane (2006) and Minas (2006) concorded on three main consecutive strategies, mass vaccination (animals of all age), vaccination of young small ruminants combined with test–slaughter of adults, and finally test and slaughter only, depending mainly on the prevalence from the highest level (> 5–10% flocks infected) to the lowest (< 1%), respectively. Therefore, valuable parameters such as prevalence of brucellosis and other epidemiological and socio-economic aspects should be considered in the way of drawing up the optimal control strategy in each area (Pérez-Sancho et al. 2015). Accordingly, epidemiological and bacteriological data are vital in control and eradication planning of the disease. Several studies related to brucellosis in many species and in different parts of Algeria were conducted (Aggad and Boukraa 2006; Bachir-Pacha et al. 2009; Lounes et al. 2014, 2021; Abdelhadi et al. 2015; Gabli et al. 2015; Kardjadj et al. 2016; Derdour et al. 2017; Kardjadj 2018; Rabehi et al. 2018; Kaaboub et al. 2019).

Nevertheless, epidemiology of brucellosis in sheep and goats was poorly studied despite its common hostage of *B. melitensis*, the most pathogenic species of humans. In addition, serological survey of small ruminant brucellosis and risk factors with an appropriate sampling design taking into consideration the interference of vaccination on serological testing and the imperfection of the later has never been investigated.

The main objectives of the present study were (1) to provide unbiased estimate of the prevalence of brucellosis in small ruminants flocks, (2) to identify risk factors associated to brucellosis at flock level, and (3) to draw up a geographic map of brucellosis distribution in small ruminants in El Oued area.

Materials and methods

Study area and animal populations

El Oued is a Saharan province located in the southeast of Algeria (Fig. 1a); it occupies an area of 34,753 km^2 and divided into 22 municipalities (Fig. 1b). It is located at an altitude of 88 m above sea level, latitude 33°21 N and longitude 6°51′ E. The southern part is covered by sand dunes, whereas the northern part is characterized by sandy desert with scarce vegetation and salt lake at the west (chott) (DPBM El Oued 2021). The climate in El Oued area is hyper-arid. Data from the Guemar weather station revealed a mean annual rainfall of 70 mm and a mean annual temperature of 28.4 °C (Bouselsal and Saibi 2022). Bouselsal and Saibi (2022) calculated the mean annual evapotranspiration to be about 1164 mm, with a high of 216 mm in July and a low of 216 mm in January (9.4 mm). According to the data of El Oued Agricultural Services (DSA El Oued 2020), there were about 1,055,027 of livestock, including 93% of small ruminants.

Sampling strategy and sample size

Between February 2019 and February 2021, a cross-sectional study was conducted using simple random sampling strategy. The primary sampling unit was the herd of small ruminant of all types of production system in El Oued area. The secondary sampling unit was the animals (sheep or goats).

The sample size to estimate the true herd-level prevalence of brucellosis was calculated according to Humphry et al. (2004), due to the imperfection of serological tests used in screening and diagnosis of brucellosis, using the following formula:

$$HN = \left(\frac{Z(C)}{L}\right)^2 \times \frac{[(HSENS(HTP) + [1 - HSPEC][1 - HTP]) \times (1 - HSENS(HTP) - [1 - HSPEC][1 - HTP])]}{(HSENS + HSPEC - 1)^2}$$

Therefore, in order to estimate a true prevalence with an imperfect test, the number of herds to be sampled (HN) was calculated using the online Epitools Calculator (Sergeant 2018), where the assumed true herd prevalence (HTP) was 3.33% (Kardjadj et al. 2016). The herd sensitivity (HSENS) and the herd specificity (HSPEC) were each chosen to be 95% with 90% confidence limits (C) (Z(C)) = 1.645) and the absolute precision (L) of 7%. Afterward, the number of animals to be sampled from each herd to achieve the HSENS and HSPEC chosen in stage one for freedom testing

was calculated using the FreeCalc application in the online Epitools Calculator (Sergeant 2018), according to the methods described by Cameron and Baldock (1998) and Cameron (1999), using an approximation to the hypergeometric distribution. The parameter inputs used were the herd size (120), test sensitivity (se) (99%) for Rose Bengal (RBT) and indirect ELISA (iELISA) tests used in parallel testing (Minas et al. 2007), test specificity (sp) (99%) for the complement fixation test (CFT) used for confirmation, the minimum within herd prevalence (35%) (Musallam et al. 2015), and the maximum acceptable type I (0.05) (1-herd sensitivity) and type II (0.05) (1-herd specificity) error values. The main outputs were the sample size (12), the cut-point number of positives in each herd (01), the herd sensitivity (HSENS) (0.9662), and herd specificity (HSPEC) (0.9938). The number of herds to be sampled was distributed proportionally to the size of small ruminants in each municipality from 22 municipalities, obtaining 51 herds after rounding to integers. The number of sheep and goats per municipality was obtained from El Oued Agricultural Services (DSA El Oued 2018). Herds and animals to be drawn from were selected randomly. Only animals more than 6 months were sampled. Verbal consent of livestock owners regarding questionnaire administration and collection of biological samples was obtained.

Sera and data collection

Blood samples were withdrawn from jugular vein by venipuncture in 5 ml labeled vacutainer tubes. Sera were recuperated in Eppendorf tubes after centrifugation at 3000 rpm for 5 min and stored at − 20 °C until their analysis. A structured questionnaire including mostly closed-ended questions was administrated to collect information regarding the socioeconomic status of the owner, the identification, the location, the composition, the characteristics, and the health history and management of the herd. These questions constituted the main herd-related variables supposed to be associated to brucellosis seropositivity.

Serological tests

All sera were subjected to two serological tests (RBT and iELISA). The Rose Bengal test (RBT) was conducted according to the fabricant's instructions (Lillidale Diagnostics ®, Dorset, UK). The indirect ELISA (iELISA) test performed to detect antibodies against *B. abortus*, *B. melitensis*, or *B. suis* by using a purified *Brucella* lipopolysaccharide (LPS) and a conjugate IgG anti-multispecies. The technique was conducted according to manufacturer instructions (ID-VET®, Montpellier, France). Positive sera to both tests were tested by the complement fixation test (CFT), which was performed according to the recommendation of the OIE (OIE 2018) and the manufacturer instructions (ID-VET ®, Montpellier, France). These techniques were performed at the Regional Veterinary Laboratory of El Oued province and the Management of Animal Health and Productions Laboratory, Institute of Veterinary Sciences, University of Frères Mentouri Constantine 1, Constantine, Algeria.

Data management and analysis

RBT and iELISA were applied in parallel. CFT was applied in series to both previous tests. Therefore, each serum sample revealed positive to all serological tests was considered positive. The herd is defined as positive when at least one sampled animal tested positively.

The true herd seroprevalence (HTP) and true individual prevalence (TP) were calculated according to the formula,$\mathrm{HTP} = \frac{\mathrm{HAP+HSPEC-1}}{\mathrm{HSENS+HSPEC-1}}$ and $\mathrm{TP} = \frac{\mathrm{AP+sp-1}}{\mathrm{se+sp-1}}$, respectively (Rogan and Gladen 1978), using the online Epitools Calculator (Sergeant 2018), where HAP is the herd apparent seroprevalence of brucellosis and AP is the individual apparent seroprevalence. Blaker method was used to estimate 95% confidence intervals of the true prevalence as described by Reiczigel et al. (2010). HSPEC and HSENS were obtained from the outputs of FreeCalc application explained in sample size calculation section.

A choropleth map was drawn to illustrate the spatial distribution of small ruminant herd prevalence of brucellosis in El Oued province per municipality. The map was created using ArcGIS 10.8.1 (ESRI 2020).

Questionnaires were inserted into Epinfo™ 7.2.3.0 (CDC 2019) software and saved afterward as Excel spreadsheet format. Subsequently, data were cleaned and coded.

Initially, explanatory analysis of each independent variable was conducted using simple logistic regression with seropositivity status of each herd as the dependent variable. Afterwards, selection of variables to be kept for the multivariable analysis was based on p value ($p \leq 0.25$) and biological plausibility. Collinearity was assessed by Cramer's V test (V coefficient > 0.15) (Akoğlu 2018). The less biologically plausible factor among collinear variables was removed. A binary logistic regression model was carried out using a backward stepwise likelihood ratio test procedure with cut-off 0.05 for entry and 0.1 for removal at each step. The evaluation of the goodness of fit of the model was performed by Hosmer and Lemeshow test. Confounding factors were revealed if there was a change in log-odds by a factor of 20% when removed, and interaction effect between variables was verified for any significance ($p < 0.05$). Variables with $p < 0.05$ at the final step were considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics 25 (IBM Corp., NY, USA).

Results

A total of fifty-one herds was investigated, in each 12 animals were sampled to constitute 612 of goats and sheep. Fourteen herds (14/51) revealed at least one seropositive animal using RBT and iELISA for screening and CFT as a confirmatory test. The true herd prevalence was 27.95% (95% CI, 17.18–42.01).

At the individual level, thirty animals (30) (sheep and goats) were seropositive to the three serological tests. The true individual seroprevalence was 3.98% (95% CI, 2.51–6.03).

Nine (9) municipalities from twenty-two (22) appeared to be seropositive to brucellosis. Three municipalities (Hamraia, Debila and Magrane) revealed 100% of brucellosis seroprevalence among sampled small ruminant herds. The chief town of El Oued and Taleb Larbi disclosed 50% of seroprevalence. Ben Guecha located at the Tunisian border showed a prevalence of brucellosis about 40%. Nakhla and Douar El-Ma located at the southern border of the province revealed 33% and 20%, respectively, of *Brucella* spp. seroprevalence. Lastly, Hassi khalifa showed a seroprevalence of about 17%, whereas the remaining municipalities revealed an absolute absence of *Brucella* spp. seropositivity. The spatial distribution of *Brucella* spp. infection in small ruminant flocks per municipality is visualized in Fig. 1b.

Three (3) variables, abortion history, poultry presence in the farm, and herd owner work time, showed p value ≤ 0.25 at the univariate analysis (Table 1); therefore, they were selected to be included into the multivariable analysis. The final binary logistic regression model showed that abortion history ($p = 0.03$) increased five (5) times the odds of *Brucella* spp. seropositivity in small ruminants herds (OR, 6.25;

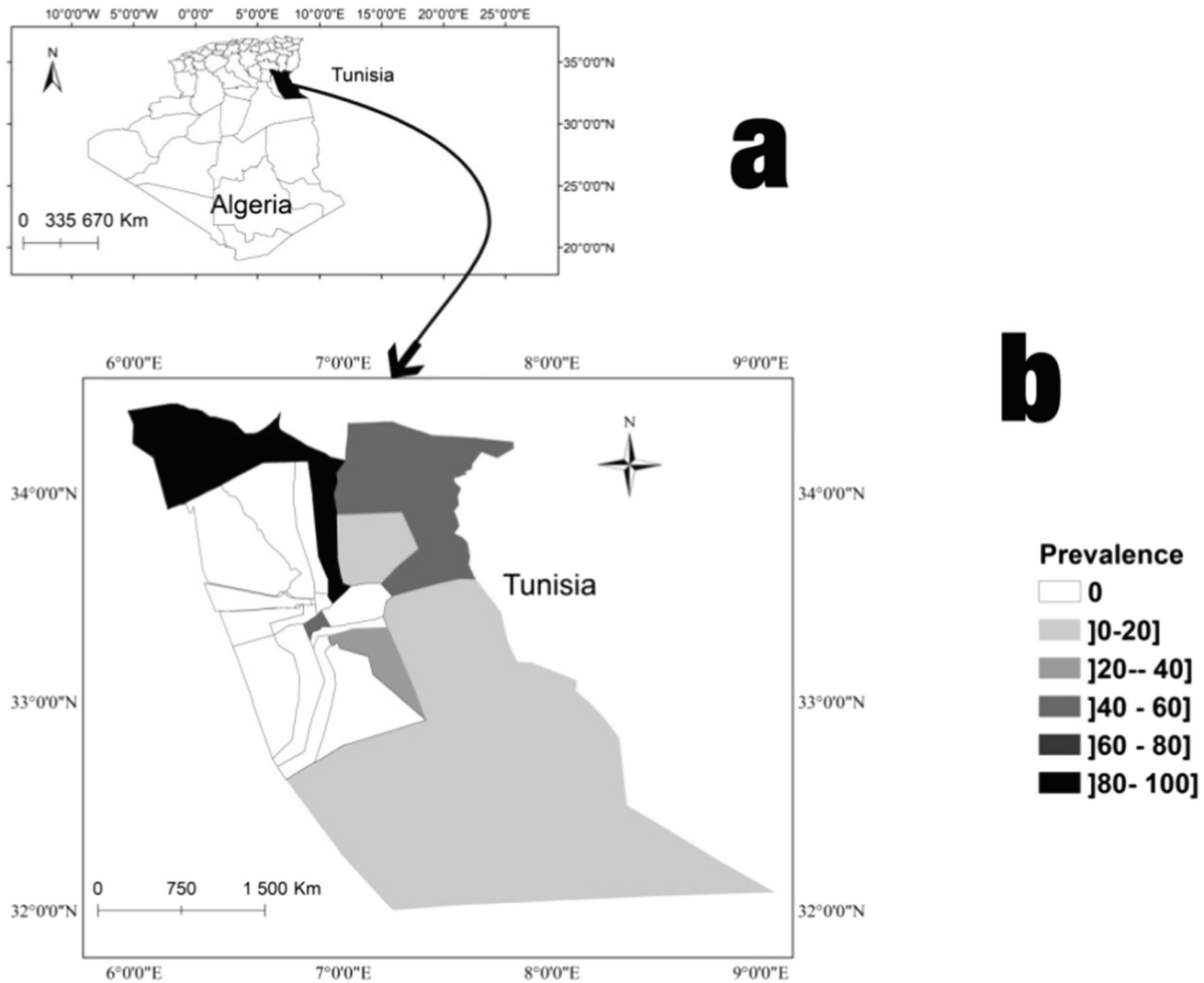

Fig. 1 Choropleth map representing the location of the study area in Algeria (**a**) and the distribution of the true prevalence of Brucellosis in small ruminant herds per municipality in El Oued province, Algeria (**b**)

Table 1 Results of association between potential herd-level risk factors and small ruminant herd *Brucella* spp. seropositivity status at the univariate analysis

Variable	Category	No. positive/total	*p* value
Herd owner work time	Full time	4/26	0.06*
	Part time	10/25	
Production system	Intensive	10/40	0.59
	Semi-extensive	2/7	
	Agro-pastoral	2/4	
Herd composition (mixed herd: animals from different origin)	Unified	12/39	0.35
	Mixed	2/12	
Herd size	<100	9/29	0.51
	≥ 100	5/22	
Production type (commercial: bought to be re-put in the market for better price)	Traditional	13/47	0.91
	Commercial	1/4	
Camels	Yes	2/10	0.56
	No	12/41	
Cattle	Yes	1/6	0.54
	No	13/45	
Sheep only	Yes	1/2	0.48
	No	13/49	
Goats only	Yes	2/5	0.51
	No	12/46	
Sheep and goats	Yes	11/44	0.33
	No	3/7	
Dog	Yes	4/21	0.27
	No	10/30	
Poultry (pigeons and chicken)	Yes	4/28	0.03*
	No	10/23	
Donkey	Yes	1/4	0.91
	No	13/47	
Abortion [a]	Yes	10/24	0.05*
	No	4/25	
Retained placenta [a]	Yes	2/6	0.78
	No	12/43	
Stillbirth [a]	Yes	11/34	0.38
	No	3/15	
Contact with other herds	Yes	4/15	0.94
	No	10/36	
Contact with wild animals	Yes	4/14	0.91
	No	10/37	
Abortion management [a]	Burn	2/2	0.50
	Burying	8/28	
	Public wasteland	2/15	
	Giving to dog	2/4	
Isolation of parturient [a]	Yes	5/15	0.63
	No	9/34	
Isolation of unhealthy animal	Yes	9/31	0.75
	No	5/20	
Renewing of herd (both: self-reproduction and purchase)	Self-reproduction	1/10	0.40
	Purchase	8/27	
	Both	5/14	

Table 1 (continued)

Variable	Category	No. positive/total	*p* value
Quarantine of newly introduced animals [b]	Yes	6/16	0.53
	No	7/25	
Cleaning frequency	Rarely	4/18	0.9
	Never	1/1	
	Frequently	9/32	
Frequent visitors	Yes	3/17	0.27
	No	11/34	
Reproducer male [a] (outsider: borrowed or purchased)	Outsider	9/28	0.52
	Raised	5/21	

*$P \leq 0.25$ were included into multivariate analysis

[a]Herds containing only males for fattening purpose and temporary herds for commercial use (lacking reproduction history) were considered missing values

[b]Herds adopting self-reproduction (not concerned by quarantine) were considered missing values

95% CI, 1.2–32.46) (Table 2). It also revealed that poultry presence among sheep and goats flock ($p = 0.01$) reduced about 89% the risk of being seropositive (OR, 0.11; 95% CI, 0.02–0.61) (Table 2). The final model fitted well the data at df = 6 (Hosmer and Lemeshow test, $X^2 = 2.92$; $p = 0.82$).

Discussion

Several serological tests have been developed for the diagnosis of *Brucella* spp. infection in different animal species (Nielsen and Yu 2010). However, all tests have limitations leading to a decrease in performance due to their ability to detect a specific isotype of antibody, cross-reaction with other bacteria, prozone phenomenon, and interference of vaccine antibodies. Therefore, a combination of tests has been selected in the present study to ensure an optimum sensitivity and specificity. RBT and iELISA are very sensitive for the detection of anti-*Brucella* antibodies in small ruminants, whereas CFT is very specific but less sensitive than the former tests (OIE 2018). Thus, RBT and iELISA were conducted in parallel to increase the sensitivity, both in series with CFT. Furthermore, as stated by the World Organization for Animal Health (OIE) (2018), RBT, iELISA, and CFT are the recommended methods for ascertainment of herd/flock prevalence of brucellosis. According to the conclusions of Stournara et al. (2007), most available serological tests used for the diagnosis of *Brucella* spp. infection could be used 11 months after brucellosis vaccination of small ruminants without diagnostic interferences. Therefore, the absence of vaccination in Algeria since 2017 supported the choice of these serological tests.

The true herd prevalence of brucellosis in small ruminants found in the present study (27.95%) was significantly higher than estimates obtained in previous studies in Setif and Batna (15.84%) (Gabli et al. 2015) and at the countrywide level (3.33%) by Kardjadj et al. (2016). This high prevalence estimate may be explained by several factors, such as the cessation of vaccination of small ruminants despite the low coverage rate, the study area being bordering province, and large part of herds adopting transhumant-grazing system near the Tunisian border, the study design, and the serological tests used for detection of *Brucella* spp. In comparison with neighboring countries, our prevalence estimate was similar to estimates found in Tunisia in sheep (21.8%) (Barkallah et al. 2017), in Egypt (20%) (Hegazy et al. 2016), and in Mali in small ruminants (25.2%) (Traoré et al. 2021). Management system livestock and communities lifestyle are common among those bordering countries. Moreover, trespassing of animals across the borders, which is challenging to control due to ruggedness and vast area

Table 2 Results of multivariate regression analysis of herd-level risk factors for small ruminant herd serological status against *Brucella* spp

Variable	*B*	SE	Wald	OR	95% CI	*p* value
Abortion history	1.83	0.84	4.75	6.25	1.20–32.46	0.03
Poultry presence	−2.18	0.85	6.5	0.11	0.02–0.61	0.01

Model − 2 log likelihood: 43.33, $X^2 = 15.3$, $p = 0.02$

B, log-odds; *SE*, standard error; OR, odds ratio; *CI*, confidence interval of odds ratio

of land, contributes to spread and maintenance of diseases. However, seroprevalence estimates in Jordan (34.3%), and Kuwait (89%) were notably higher than our results (Musallam et al. 2015; Al-Sherida et al. 2020). Mussalam et al. (2015) ascribed the high prevalence to limited control efforts, uncontrolled movement of animals, and itinerant or semi-nomadic farming system.

As expected, the highest prevalence estimates were reported in northern and eastern municipalities of El Oued province, sharing land borders with three provinces (Tebessa, Khenchela, and Biskra) and Tunisia where small ruminant flocks graze freely in both sides depending on season and abundance of pasture, therefore increasing the likelihood of diseases spread. In addition, three municipalities near the Tunisian border (Tableb larbi, Benguecha, and Douar El-maa) contain about 35% of small ruminant flocks. El Oued area is known as a commercial tycoon in Algeria including livestock trade, which may explain the high seroprevalence of brucellosis found in the Chief town municipality. Western municipalities showed null brucellosis seropositivity, which might be due to the increased number of fattening farms of young animals in this area and the lack of livestock transhumance due to similarities of desert terrain. Due to limited resources, spatial distribution of brucellosis is advantageous in low- and middle-income countries, in the matter of targeting the highest infected regions in terms of brucellosis control tools such as test-slaughtering, vaccination, livestock movement control, and awareness campaign regarding farm biosecurity measures.

History of abortion was significantly associated to small ruminant herd seropositivity status in the multivariate analysis. Despite the susceptibility of both genders to brucellosis, the common sign of acute infection is abortion in the last trimester in sheep and goats (Xavier et al. 2010). In addition to the decrease in production and infertility as a complication, the abortion contributes also to the persistence of brucellosis in the flock. Undoubtedly, material from an abortion such as the placenta, fetuses, and fetal fluids is highly infective and represents the main source of transmission within the herd (Samadi et al. 2010).

Accordingly, our findings highlight the importance of the implementation of biosecurity measures related to abortion in farms in order to reduce brucellosis prevalence. Practices such as appropriate abortion management, isolation of parturient and/or aborted female, hygiene, and disinfection could efficiently contribute to diminish the risk of introduction and maintenance of brucellosis in small ruminants flocks even though these factors have not been statistically significant in our study. Several researchers came to the same results (Boukary et al. 2013; Edao et al. 2020; Alemayehu et al. 2021).

The presence of poultry (chicken and pigeon) was associated with seropositivity in sheep and goats herd as a protective factor. Pigeons in this area are raised as domestic animals for meat and eggs. Despite the logical explanation of the role of mechanical vector of the said birds in brucellosis transmission within the flock as stated by Roux (1979), poultries may as well provide an insect control by ingesting them. According to Coelho et al. (2015), blood-sucking insects contribute to mechanical dissemination of brucellosis as they feed on their blood, tears, and placental secretions. Moreover, It has been reported that *Brucella* was isolated from the stomach contents of *Stomoxys calcitrans*, *Ornithodoros*, and *Musca autumnalis* (stable fly) (Coelho et al. 2015).

Recently, Huang et al. (2020) isolated *B. melitensis* biotype 3 from eggs and engorged adults of *Dermacentor nuttalli* (native tick species of Inner Mongolia) and suggested that the recent prevalence of brucellosis outbreaks in the Inner Mongolia regions (China) may be attributed to an increase in the activity of ticks and other air-borne vectors. Furthermore, in an earlier study, *B. melitensis* and *B. abortus* were identified in *Dermacentor marginatus* ticks collected from sheep and cattle, respectively, whereas *Brucella* DNA was detected in eggs and larvae of *Brucella* DNA-positive engorged female ticks disclosing transovarial and transstadial transmission of *Brucella* spp. in *D. marginatus* ticks (Wang et al. 2018). Additionally, Zhang et al. (2021) demonstrated the presence of *Brucella* spp. in *Haemaphysalis longicornis* ticks. Considering these interesting previous findings, the conjectured role of ticks, fleas, or other blood-sucking insects in brucellosis infection should be considered in the study area where both animal-to-animal contact and contamination of pastures are reduced under the dry climate. Indeed, this allowed us to suggest that chicken and pigeon presence within small ruminant farms can have a protective role by feeding on insect vectors responsible for brucellosis spread.

Counterintuitively, potential risk factors such as pastoralism, commercial production type, herd size, using purchased animals for renewing, retained placentas and stillbirth history, and origin of the breeding male were not found to be associated with brucellosis prevalence in the present study. This lack of statistical association may be due to a number of factors such as the large difference between the expected prevalence (3.33%) and the estimated true prevalence (27.95%) which might have biased the sample size. Additionally, bias in cross-sectional studies related to selection and confounding as described by Pandis (2014) could be another element in addition to the subjectivity of some collected data in the questionnaire.

Based on the true herd prevalence estimate, the mass vaccination strategy of the whole flock is highly recommended in order to mitigate contamination and transmission of the disease, targeting specifically the highest prevalence areas. In addition, our findings highlight the importance of the implementation of biosecurity measures at farms to reduce the risk of introduction, establishment, and spread of brucellosis. Therefore, education programs for farmers and farm workers to implement effective biosecurity measures to prevent the risk of introducing and spreading of brucellosis or other infectious diseases are highly required in developing countries like Algeria. Diagnostic services need to be improved, and implementation of molecular biology remains necessary to boost the brucellosis control program. In consideration of disparity of livestock systems and species, environmental conditions, and social aspects across Algeria, further detailed epidemiological studies at regional scales seemed imperative in order to adjust proposed national control program to the regional data and requirement.

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Author contribution All authors contributed to the article and approved the submitted version. Nacira Ramdani contributed in study design, blood sample collection, and laboratory and data analysis and article redaction. Sabrina Boussena contributed by scientific critic, supervision of the work during the whole process, and article correction in editing. Omar Bouaziz contributed to the supply of study materials and analytical reagents. Nassim Moula contributed in scientific critic and article correction. All authors read and approved the final manuscript.

Data availability Data regarding livestock number per municipality, human cases number, and brucellosis control program are available in the government department archives mentioned in the text.

Code availability Not applicable.

Declarations

Ethics approval Verbal consent regarding blood and data collection was obtained from animal owners that were included in the present study.

Statement of animal rights Blood drawing was performed by a veterinarian with respect and preservation of animal health and welfare.

Consent to participate All co-authors have agreed to participate in conducting this study.

Consent for publication All co-authors gave their consent for the publication of the present paper.

Conflict of interest The authors declare no competing interests.

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