The risk of bovine brucellosis in Ecuador : structuring by cattle movements, risk factors for infection of farms and perception by the various actors

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Le risque de brucellose bovine en Équateur : structuration par les mouvements de bovins, facteurs de risque d'infection des élevages et perception par les différents acteurs

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«La curiosidad y el afán de resolver dilemas son el sello distintivo de nuestra especie»

Carl Sagan

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Aplicación de la metodología de análisis de redes sociales en el estudio de la propagación de enfermedades en la ganadería en Ecuador. National scientific meeting**,** Special Interest Group in One-Health, *e-Health*. By Corporación Ecuatoriana para el Desarrollo de la Investigación y la Academia CEDIA. Ministerio del Ambiente del Ecuador (MAE), Instituto Nacional para la Salud Pública (INSPI), 9 y 10 de diciembre 2021. Quito-Ecuador.

Social network analysis en el estudio de la propagación de enfermedades en la ganadería bovina. II Congreso internacional de producción pecuaria, Universidad Politécnica del Chimborazo ESPOCH, Facultad de Ciencias Pecuarias, 8-12 novembre 2021. On-line

Résumé

La brucellose bovine est une maladie qui provoque des pertes économiques en Équateur et met en danger la santé publique des habitants. Certains éléments, tels que les mouvements de bovins dus au commerce, le type d'exploitation, certaines pratiques d'élevage, ainsi que le niveau de connaissance et la manière d'appréhender la maladie de la part de l'éleveur peuvent entraîner la propagation de la brucellose et maintenir des taux de prévalence élevés dans différentes régions du pays. Le premier objectif de cette thèse était de comprendre comment une infection telle que la brucellose peut se propager à travers un réseau national de commerce de bovins. En utilisant la base de données des mouvements de bovins de l'Équateur pour les années 2017 et 2018, nous avons construit deux réseaux de mouvements : le premier pour une maladie à propagation rapide telle que la fièvre aphteuse due à un sérotype qui serait absent dans le vaccin utilisé actuellement dans le pays, et un autre réseau pour représenter la propagation d'une maladie à propagation lente telle que la brucellose bovine. Les réseaux ont été analysés annuellement et mensuellement au niveau des paroisses en utilisant la méthodologie de l'analyse de réseaux. Pour les deux réseaux, la plus grande composante fortement connexe au niveau annuel comprenait 90 % des nœuds et la plus grande composante faiblement connexe tous les nœuds, ce qui indique une très faible fragmentation. Une analyse de percolation a révélé que la plupart des paroisses devaient être supprimées pour éliminer les plus grandes composantes fortement connexes. Sur la base de certaines caractéristiques du réseau, nous avons pu établir qu'une maladie hautement contagieuse pouvait se propager rapidement dans tout le pays et qu'une infection à transmission plus lente, telle que la brucellose bovine, pouvait se propager au sein de groupes locaux de paroisses. Le deuxième objectif de la thèse était d'estimer la prévalence inter-exploitation, les facteurs de risque de la brucellose bovine et les pratiques à haut risque pour la santé humaine dans les petites et moyennes exploitations bovines d'une région tropicale de l'Équateur. La prévalence apparente dans les élevages était de 11,5 % (IC 95 % : 6,7-16,2). Le groupe d'exploitations de taille moyenne avait une prévalence significativement plus élevée que les petites exploitations. Deux analyses multivariées ont été réalisées pour identifier les facteurs de risque associés à la brucellose bovine ou aux problèmes de reproduction. La taille de l'exploitation a été identifiée comme un facteur de risque pour la brucellose bovine et l'incinération/l'enfouissement du matériel d'avortement comme un facteur de protection. La taille de l'exploitation et la positivité de la brucellose ont été identifiées comme des facteurs de risque pour les problèmes de reproduction. Le troisième objectif de cette étude était d'appliquer la méthode de recherche qualitative des groupes de discussion aux producteurs de petites et moyennes exploitations et aux vétérinaires des secteurs public, privé et universitaire afin de mieux comprendre les aspects sociaux liés à la présence éventuelle de la maladie dans les petites et moyennes exploitations. Nous avons constaté que le niveau de connaissance et de sensibilisation à la maladie chez les petits et moyens éleveurs ainsi que des éléments liés à leur éducation, âge et sexe seraient en train d'affecter la mise en œuvre des mesures de contrôle de la brucellose bovine en Équateur.

Mots clés: analyse des réseaux, brucellose, Équateur, groupes de discussion, mouvements de bovins, facteurs de risque.

Abstract

Bovine brucellosis is a disease that causes economical losses in Ecuador and puts the inhabitant's public health at risk. Some factors, such as cattle movements due to trade, the type of farm, some farming practices, the level of knowledge on bovine brucellosis and the way of understanding the disease by the farmer can lead to bovine brucellosis spreading and maintenance of high prevalence values in different regions of the country. The first objective of this thesis was to understand how an infection such as bovine brucellosis can spread through a national cattle trade network. Using the database of the cattle movements of Ecuador for the years 2017 and 2018, we built two movement networks: the first for a rapidly spreading disease such as foot-and-mouth disease due to a serotype currently absent in the vaccine used in the country, and the other for a network representing the spread of a slow-spreading agent such as bovine brucellosis. The networks were analyzed annually and monthly at the parish level using the network analysis methodology. For both networks, the largest strong component at the annual level included 90% of the nodes, and the largest weak component had all nodes, indicating very low fragmentation. A percolation analysis revealed that most parishes needed to be removed to make disappear the largest strong component. Based on some network characteristics, we established that a highly contagious disease could spread rapidly and that a slower-transmitting infection, such as bovine brucellosis, could spread within local groups. The second objective of the thesis was to estimate the between herd prevalence and risk factors of bovine brucellosis and high-risk practices for human health in small and medium-sized cattle farms in a tropical region of Ecuador. The apparent prevalence among farms was 11.5% (95% CI: 6.7-16.2). The medium-sized group of farms had a prevalence significantly higher than the small farms. Two multivariable analyses were conducted to identify risk factors associated with bovine brucellosis or reproductive problems. Farm size was identified as a risk factor for bovine brucellosis and incineration/burial of abortion material as a protective factor. Farm size and brucellosis positivity were identified as risk factors for reproductive problems. The third objective of this study was to apply the qualitative research method of focus groups in small and medium scale farmers and veterinarians from the public, private and academic sectors to achieve a better understanding of social aspects related to the possible presence of the disease in small and medium-sized farms. We found that the level of knowledge and awareness of the disease among small and medium scale farmers and other factors related to education, age and gender, which could be affecting the implementation of measures for the bovine brucellosis control in Ecuador.

Key words: brucellosis, cattle movements, Ecuador, focal groups, network analysis, risk factors.

Résumé détaillé

La brucellose bovine est une maladie qui affecte un large éventail d'animaux hôtes dans le monde entier. Elle a été reconnue comme l'une des zoonoses bactériennes les plus importante au monde (Corbel, 1997; Osorio FJ, 2004). La bactérie *Brucella abortus* peut entraîner des pertes chez plusieurs espèces d'intérêt productif (bovins, caprins, ovins et porcins) (Galińska & Zagórski, 2013). Les pertes économiques sont considérables en raison des avortements, de la prolongation des jours ouverts ou sans gestation, et des restrictions commerciales sur les animaux et les produits infectés (Olsen & Tatum, 2010). Chez les espèces laitières, le lait est contaminé à vie, ce qui oblige l'éleveur à éliminer et à abattre les femelles infectées, avec un coût énorme lié aux efforts déployés pour éliminer la maladie dans l'exploitation (Cárdenas et al., 2019; Laplume et al., 2013; Nicoletti, 1980).

La brucellose est une des zoonoses les plus répandues au monde, avec autour de 500 000 nouveaux cas par an (Godfroid et al., 2005 ; Eales et al., 2010). La présence de symptômes de brucellose chez les personnes affectées réduit leur capacité de travail, et elles sont obligées d'engager des frais de soins médicaux et de traitement (Corbel, 1997). La brucellose est considérée comme une maladie professionnelle car elle touche une grande partie des personnes qui sont en contact avec des animaux infectés, comme les éleveurs, les vétérinaires, les employés des abattoirs (Gwida et al., 2010; Tschopp et al., 2022), et même le personnel de laboratoire qui peut s'infecter accidentellement (Agrocalidad, 2008).

L'élevage bovin en Équateur est un pilier fondamental du secteur agricole. Le développement de l'élevage a favorisé la production de denrées alimentaires telles que la viande et le lait, qui sont essentielles à la sécurité alimentaire de la population, ainsi que pour l'approvisionnement en matières premières pour la production d'autres biens de consommation. L'élevage fait également appel à la main d'œuvre paysanne, ce qui contribue au dynamisme de l'économie rurale. La présence enzootique de la brucellose en Équateur n'entraîne seulement des pertes dans ce secteur de l'élevage, elle constitue également une menace pour la santé publique. On estime que la brucellose bovine entraîne des pertes annuelles de 5,5 millions de dollars dans le pays en raison des avortements, de la réduction de la production de lait, de la confiscation et de l'abattage des animaux positifs (Agrocalidad, 2008).

Bien que des études aient été menées pour établir la prévalence de la brucellose bovine dans plusieurs régions du pays, les aspects susceptibles de faciliter sa propagation ou d'entraver la mise en œuvre efficace d'un programme de contrôle, tels que les mouvements de bovins et la manière dont les agriculteurs traitent la maladie, n'ont pas encore été analysés en Équateur. Dans ce contexte, les principaux objectifs de cette thèse étaient d'étudier l'implication potentielle du réseau

de commerce des bovins dans la propagation de la brucellose bovine en Équateur et le rôle joué par les petits et moyens éleveurs dans le maintien de la maladie.

Le commerce des animaux et des produits en Équateur s'effectue sur l'ensemble du territoire national grâce à un réseau routier qui relie les 23 provinces du pays et permet d'atteindre les endroits les plus éloignés dans la même journée. Jusqu'à présent, en Équateur, les mouvements de bovins ne sont autorisés qu'avec un certificat de vaccination contre la fièvre aphteuse. Le commerce des animaux peut contribuer à la propagation de maladies infectieuses telles que la brucellose, car il facilite le déplacement d'animaux infectés d'une région à l'autre. Dans ce contexte, trois questions se posent : *i)* est-il possible de structurer un réseau représentant le commerce des bovins en Équateur et permettant de comprendre ses principales caractéristiques, telles que la taille, la structure et la connectivité ? *ii)* un réseau représentant le commerce des vaches pourrait-il aider à comprendre la propagation de la brucellose bovine ? *iii)* une élimination sélective des nœuds de ces réseaux pourrait-elle aider à identifier la manière la plus appropriée de contrôler la propagation d'une maladie ?

D'autre part, le secteur de l'élevage bovin en Équateur est composé de différents systèmes de production qui se distinguent par leur spécialité productive, leur taille et leur utilisation de la technologie, et éventuellement par le niveau d'information et de sensibilisation des producteurs à l'égard de la brucellose bovine. Sur l'ensemble des exploitations agricoles enregistrées en Équateur, environ 76 % correspondent à des unités de moins de 10 bovins (INEC, 2008). Cependant, la quasi-totalité des études concernant la brucellose bovine ont été réalisées dans des exploitations de plus grande taille. On sait très peu de choses sur le rôle joué par les petits et moyens éleveurs de bovins dans le maintien et la diffusion de la brucellose bovine en Équateur et principalement dans certaines provinces où très peu d'études ont été menées. Dans cette perspective nous nous demandons: *i)* quelle est la prévalence de la brucellose dans les exploitations appartenant à ces catégories d'éleveurs ? *ii)* quel est le niveau de connaissances de ces éleveurs sur la maladie ? *iii)* existe-t-il des pratiques de gestion de l'élevage ou des produits animaux consommés ou vendus dans ces exploitations qui peuvent constituer une menace pour la santé publique ? Pour approfondir les questions sociales liées à la lutte contre la brucellose bovine, nous avons cherché à répondre aux questions sur le niveau de connaissance, de sensibilisation et de perception du risque de la brucellose bovine chez les petits et moyens éleveurs, et à recueillir les opinions des professionnels de la santé animale sur la lutte contre la maladie.

La stratégie de recherche proposée pour répondre à ces questions est basée sur trois axes décrits dans les trois chapitres de cette thèse.

Compte tenu du fait que la propagation de la brucellose entre les fermes est principalement liée aux mouvements commerciaux de bovins, nous avons utilisé la méthodologie d'analyse de réseaux pour étudier les mouvements de bovins enregistrés dans la base de données nationale entre 2017 et 2018 (qui étaient les deux dernières années disponibles), et à la plus petite échelle géographique. Ce travail est décrit dans le premier chapitre. Nous avons utilisé les données des mouvements de bovins pour construire deux types de réseaux : un réseau comprenant tous les mouvements de bovins représentant le risque de transmission d'une maladie à propagation rapide comme la fièvre aphteuse due à un sérotype qui ne serait pas inclus dans le vaccin utilisé actuellement lors de campagnes de vaccination contre cette maladie et un réseau comprenant uniquement les mouvements de vaches représentant le risque de transmission de la brucellose bovine, une maladie à évolution lente affectant principalement chez les femelles adultes. Les paroisses (les plus petites unités géographiques) ont été considérées comme les nœuds du réseau et les mouvements de bétail entre les paroisses comme les liens. Les indicateurs de réseau calculés aux niveaux annuel et mensuel étaient proches pour les deux types de réseaux. Il a été mis en évidence la forte centralité des paroisses où des marchés étaient présents, puisque la moitié des mouvements de bovins provenant des fermes étaient dirigés vers ces paroisses. En outre, les paroisses avec marchés étaient fortement connectées aux paroisses sans marchés, parfois situées dans régions éloignées du pays. Dans un scénario d'introduction de la fièvre aphteuse, les paroisses où se trouvent les marchés seraient donc les sites où les interventions de surveillance devraient être renforcées ou des marchés fermés, afin d'enrayer la propagation de cette maladie. Pour les deux types de réseaux, la plus grande composante fortement connectée (niveau annuel) incluait plus de 90 % des nœuds et la plus grande composante faiblement connectée incluait tous les nœuds, ce qui indique une très faible fragmentation. Cela implique que les deux maladies pourraient atteindre pratiquement l'ensemble du territoire national par le biais des mouvements d'animaux infectés. En outre, les caractéristiques des réseaux de petit monde ont été identifiées pour les deux types de réseau, ce qui signifie qu'une infection peut se propager localement parmi les paroisses voisines, mais aussi se propager rapidement vers des sites éloignés. Contrairement aux contrôles actuellement appliqués à la fièvre aphteuse, qui comprennent la vaccination obligatoire et le contrôle de la vaccination des bovins déplacés, dans le cas de la brucellose bovine, aucune de ces exigences n'est mise en œuvre. L'analyse des réseaux de vaches au niveau des paroisses a permis d'identifier des communautés de paroisses correspondant aux régions où le commerce des vaches est le plus intense. L'identification de ces régions pourrait constituer un outil pour la conception et la mise en œuvre de plans de contrôle qui viseraient à constituer des zones exemptes de brucellose avec vaccination. Enfin, une analyse de percolation a montré que la plupart des paroisses devraient être supprimées pour réussir à fragmenter les plus grandes composantes fortement connectées. Toutes ces caractéristiques devraient être prises en compte lorsqu'il s'agit d'envisager des mesures de prévention en Équateur dans le cas d'une maladie émergente comme la fièvre aphteuse ou des mesures de contrôle pour une maladie endémique comme la brucellose.

Le deuxième chapitre correspond à une étude de la prévalence et des facteurs de risque de la brucellose bovine dans des petites et moyennes exploitations bovines menée dans une zone de la province d'Esmeraldas, une région tropicale de l'Équateur. Au total, 173 échantillons de lait de tank ont été prélevés et analysés au moyen d'un test ELISA indirect. Une enquête a également été menée auprès de chaque éleveur afin de recueillir des informations sur la gestion du troupeau, d'évaluer le niveau de connaissance de la maladie et d'estimer le risque que l'apparition de la brucellose dans ces exploitations pourrait représenter pour la santé publique. La prévalence apparente dans les exploitations était de 11,5 % (IC 95 % : 6,7-16,2 %). Le groupe d'exploitations de taille moyenne avait une prévalence significativement plus élevée (23,8 %, IC 95 % : 10,9 - 36,6 %, $p < 0,0001$) que le groupe de petites exploitations (7,6 %, IC 95 % : 4,5-9 %). Deux analyses multivariées ont été réalisées pour identifier les facteurs de risque associés à la brucellose bovine ou aux problèmes de reproduction au niveau de la ferme. La taille de l'exploitation a été identifiée comme un facteur de risque de positivité de la brucellose bovine (OR : 3,7, 95% CI : 1,39 - 9,84, $p = 0,008$) et l'incinération/enfouissement du matériel d'avortement comme un facteur de protection (OR : 0,4, 95% CI : 0,14 - 0,98, $p = 0,04$). La taille de l'exploitation et la positivité de la brucellose ont été identifiées comme des facteurs de risque pour l'apparition de problèmes de reproduction. Seuls 25 % des éleveurs connaissaient la brucellose bovine. Du lait caillé non pasteurisé était régulièrement consommé et commercialisé dans 112 exploitations, dont 14 étaient positives à la brucellose bovine. Des pratiques à haut risque, telles que la manipulation de fœtus avortés, ont également été signalées. Ces résultats indiquent qu'il est nécessaire de développer des programmes d'éducation à la santé publique ciblant les petits et moyens producteurs en Équateur.

Le troisième chapitre correspond à une étude qualitative menée à l'aide de la méthodologie des groupes de discussion. Nous avons cherché à établir le niveau de connaissance, de sensibilisation et de perception de la brucellose dans un groupe de discussion composé d'éleveurs de fermes de petite taille et dans un groupe focal composé d'éleveurs de fermes de taille moyenne. Deux groupes de discussion composés de vétérinaires ont également été réunis afin d'étudier la perception des professionnels de la santé animale sur la situation de la brucellose sur le terrain et les éléments qui pourraient entraver la mise en œuvre du programme de contrôle de la brucellose en Équateur. L'étude nous a permis d'établir les faibles niveaux de connaissance et de sensibilisation à la brucellose parmi les producteurs des deux groupes, ainsi qu'une série d'éléments liés à l'éducation, aux traditions et à la culture qui pourraient constituer des pratiques à risque pour la présence de la maladie. Certaines conditions telles que le niveau d'éducation, le sexe et l'âge pourraient restreindre l'accès à l'information et affecter l'inclusion de beaucoup d'éleveurs dans le plan de contrôle de la brucellose bovine. Les groupes de discussion de vétérinaires ont évoqué l'incorporation de l'industrie laitière et la création de zones exemptes de brucellose avec vaccination, dans le cadre du plan de contrôle.

Notre objectif initial était d'effectuer une analyse de réseaux au niveau de l'exploitation agricole. Cependant, après avoir examiné les bases de données, nous avons réalisé l'étude au niveau de la paroisse car nous avons trouvé des incohérences entre les bases de données. Les futures études à mener sur les mouvements de bovins mentionnés ci-dessus devraient reposer sur des données plus précises. Pour cela, les bases de données disponibles dans les services vétérinaires doivent être améliorées. Dans le cadre de la lutte contre la brucellose bovine, les exploitations qui participent au programme "ferme indemne de brucellose bovine" pourraient également être identifiées sur une base annuelle. Les incohérences des bases de données identifiées dans notre travail seront signalées aux services vétérinaires officiels.

Pour répondre au manque d'informations sur la présence de la brucellose chez les petits et moyens agriculteurs, nous avons utilisé deux outils : une analyse des facteurs de risque pour identifier les facteurs associés à la présence de la maladie, et une étude qualitative par le biais d'un groupe de discussion composé de petits agriculteurs et d'un autre composé d'agriculteurs moyens.

Nous avons jugé essentiel de mener l'enquête sur les facteurs de risque dans une région où la prévalence de la brucellose est élevée et où aucune étude n'avait été réalisée auparavant. Nous avons constaté que les exploitations de taille moyenne présentaient des valeurs de prévalence significativement plus élevées, des probabilités plus élevées d'être infectées que les petites exploitations en raison de la taille de l'exploitation et du type de gestion des avortements. Les problèmes de reproduction étaient également plus fréquents dans ce groupe d'exploitations. Ces résultats suggèrent qu'un grand nombre d'éleveurs d'exploitations moyennes pourraient être dans un processus de transition vers un schéma commercial qui se reflète dans l'augmentation de la taille de leurs troupeaux et dans la fréquence des échanges commerciaux d'animaux. Ces augmentations ne vont apparemment pas de pair avec les processus de formation en matière de gestion de la reproduction et de la santé qui pourraient leur permettre de faire face aux défis liés à la brucellose bovine. Ainsi, le faible niveau de connaissance de la maladie dans les deux groupes d'éleveurs pourrait contribuer à la persistance de la maladie dans la région. Cette circonstance a été soulignée par les participants aux groupes de discussion des vétérinaires et est conforme aux résultats obtenus dans les groupes de discussion des éleveurs. Bien que les deux types d'élevages soient à risque, il faut considérer que les moyens éleveurs effectuent comparativement plus de transactions commerciales et déplacent un plus grand nombre d'animaux vers et depuis leurs fermes, augmentant ainsi la probabilité d'échanger des animaux infectés.

Dans nos études, nous avons constaté que les moyens et surtout les petits éleveurs n'ont pas la même possibilité que les éleveurs possédant des fermes d'une technicité plus élevée de recevoir une incitation, par le biais d'intermédiaires, à commercialiser des produits libres de brucellose. Pourtant, en Équateur, il existe quelques expériences dans lesquelles le secteur privé a encouragé le contrôle de la brucellose en favorisant les achats de produits par l'intermédiaire d'associations de petits et moyens producteurs. Pour comprendre les mécanismes possibles à appliquer pour obtenir la participation de ce type d'éleveurs, nous proposons d'étendre l'étude des groupes de discussion aux éleveurs industriels, à l'industrie laitière et aux petits et moyens producteurs associés qui vendent actuellement à des usines de transformation.

Certaines pratiques à risque identifiées dans l'étude d'Esmeraldas, tels que la consommation de lait cru ou la consommation d'avortons bovins, ont également été mises en évidence dans les groupes de discussion composés de vétérinaires et d'éleveurs.

L'analyse qualitative a permis d'identifier des questions qui ne pouvaient être mises en évidence que par cette technique, ce qui montre qu'il s'agit d'un outil qui devrait être utilisé plus fréquemment dans le cadre des études épidémiologiques afin d'obtenir une image complète d'une situation. La méthodologie des groupes de discussion nous a permis d'identifier le fait que les petits éleveurs n'ont pas accès à l'information sur la brucellose et qu'ils ignorent les stratégies actuellement utilisées pour la contrôler. Leur niveau de sensibilisation et leur perception du risque sont faibles. Cela les conduit à mettre en œuvre des pratiques à risque pour la transmission de la brucellose aux animaux et à l'homme. Des programmes d'éducation et de sensibilisation des éleveurs pourraient contribuer à améliorer l'efficacité et la durabilité du plan de lutte contre la brucellose, comme l'ont suggéré les participants aux groupes de discussion de vétérinaires.

La brucellose bovine en Equateur reste donc un problème multifactoriel qui comprend différents aspects qui doivent être abordés tels que le commerce de bovins, les activités humaines liées à la gestion des animaux et des facteurs socioculturels. Les méthodes épidémiologiques et l'analyse sociologique que nous avons appliquées dans ce travail de thèse, nous ont permis d'améliorer la compréhension de différents aspects de la brucellose bovine en Equateur, d'évaluer le risque associé aux mouvements de bovins, d'identifier les facteurs de risque de la présence de la maladie chez des petits et moyens éleveurs, et leur façon de percevoir le risque de la présence de la maladie. Néanmoins, de nombreuses questions doivent encore être mieux comprises et nous espérons que les résultats que nous avons obtenus pourront être utiles aux services vétérinaires nationaux et aux éleveurs de l'Équateur.

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GENERAL INTRODUCTION

1. General concepts of brucellosis

Brucellosis is a disease that affects a wide range of animal hosts worldwide. It has been recognized as an important bacterial zoonoses worldwide (Corbel, 1997; Osorio FJ, 2004). During the 19th century, brucellosis was known as Malta or Mediterranean fever due to an outbreak occurred in the English troops while they were on the island of Malta. This disease was also called undulant fever due to the symptoms caused in humans (Gaucy-Amato, 1995; Wasee Ullah et al., 2013). The bacterium that causes the disease bears the name of its discoverer (David Bruce), who isolated it for the first time in 1886 (Godfroid et al., 2011; A. El-Sayed & Awad, 2018).

Many countries have made significant progress in controlling the disease in domestic animals. Several countries in Western and Northern Europe, Canada, Japan, Australia and New Zealand are considered bovine brucellosis free (WOAH, 2023). On the other hand, a high incidence of the disease has been reported in the Middle East, Mediterranean, sub-Saharan Africa, China, India, and the Caribbean and South American countries (Ferreira Neto, 2018; Corbel, 2006).

In some countries, the lack of knowledge and the low level of awareness of farmers about brucellosis significantly increases the seropositivity of brucella infection in animals (Deka et al., 2018). Furthermore, the presence of brucellosis in humans is closely related to the presence of the disease in domestic animals. Therefore, the control of the disease in animals contributes notably to the decrease in the incidence of human cases (Vargas-Chiarella et al., 2016).

1.1. Etiology and pathogenesis

The causative agent of brucellosis is the facultative Gram-negative and intracellular coccobacillus *Brucella* spp. From a taxonomic point of view, brucellas are part of the second subdivision of proteobacteria. The genus brucella currently consists of 12 species which infect wild and domestic animals (Hull & Schumaker, 2018). Beside the six classical species that affects terrestrial mammals (*B. abortus, B melitensis, B. suis, B. canis, B. neotomae* and *B. ovis*) (WOAH, 2023), novel members isolated from marine mammals and primates have been included lately (*B. pinnipedialis, B.ceti* and *B. papionis*) (Foster G et al., 2007; Whatmore et al., 2014).

Some *Brucella* strains can infect different species of domestic and wild animals. It is the case of *B. abortus* that can affect not only cattle but also goats, sheep, dogs, pigs, horses, camels and guinea pigs (Denny, 1973; Hensel et al., 2018; Lilenbaum et al., 2007; Stableforth, 1952). Strains of *B. abortus* and *B. suis* have been isolated of wildlife mammals such as bison, elk, wild boar, European hare, fox, African buffalo, antelope, reindeer, caribou and capybara (Davis & Elzer, 2002; Godfroid, 2002; Lord & Flores, 1983). Cases of infection by *B. mellitensis*, which is more frequent in goats and sheep have been reported in cattle (Álvarez et al., 2011; Bankole et al., 2010; Cook & Noble, 1984).

Brucella infection occurs when the bacterium enters the body through the mucous membranes of the respiratory or digestive tracts. The bacterium manages to survive and avoid the activation and recognition of the host's immune system and manages to locate, survive and multiply inside the host's mononuclear phagocytes, where it acts as an intracellular parasite, adapting to the limited oxygen conditions of the cells (Aréstegui et al., 2001; Seleem et al., 2008). Macrophages and neutrophils carry the bacteria to supramammary, gastric, iliac, sublumbar, colorectal, and mandibular lymph nodes (Foster et al., 1996; Meador et al., 1989). This process leads to the spread of the bacteria to the different organs of the reticuloendothelial system, including the lungs, spleen, liver and bone marrow. Muscle is not the preferred site for *Brucella* spp. However, specific organs such as the spleen, liver, and lymph nodes are target tissues (Fero et al., 2020; González-Espinoza et al., 2021). *Brucella* show strong tissue tropism for the lymphoreticular and reproductive systems; abortion in females and infertility in males result from this characteristic. In the pregnant animal, brucella replicates within the placental chorioallantoic trophoblasts. Invasion of the placenta produces placentitis that can be acute and generalized and lead to early fetal death followed by abortion (Enright, 1990; Głowacka et al., 2018).

The bacterium achieves intracellular adherence thanks to a lipopolysaccharide that is part of the cell wall of *Brucella* spp. This lipopolysaccharide promotes adherence to host mononuclear phagocytes through mannose receptors. The cells of the placenta are rich in mannose receptors. This fact, together with the tropism for erythritol, a carbohydrate produced by the fetus, found in placental and fetal fluids, would explain *Brucella* avidity for bovine fetuses and pregnant uterus (Acha et al., 2001; Aréstegui et al., 2001; Pontow et al., 1993). On the contrary, erythritol has not been found in the placentas of guinea pigs, rats, mice, rabbits, or humans, which has served to explain the less frequent affection of the reproductive tract of these species by *B. abortus* (Samartino & Enright, 1993).

The ability of bacteria to survive intracellularly keeps them protected from immune responses and limits the action of antibiotics, which is why antibiotic treatment in cattle has been ruled out and the repertoire of antibiotics and treatment regimens in humans is limited (Alavi & Alavi, 2013; De Figueiredo et al., 2015; Moreno, 2014; Solera et al., 1998).

1.2. Modes of transmission of brucellosis

Brucella can be transmitted horizontally or vertically. The pathogen is found in the highest concentration in the uterus of pregnant animals. Aborted fetuses, placental membranes, and uterine secretions are the primary source of infection. Brucellosis is spread by vertical transmission, infecting newborn calves and lambs in utero or through milk (Khan & Zahoor, 2018; Khurana et al., 2021).

Horizontal contagion in cattle occurs through contact with cows' and bulls' reproductive secretions and excretions. Infected bulls can be lifelong sources of infection. The infection can also occur indirectly by ingesting contaminated food or water or through artificial insemination with contaminated material (Corbel, 1997).

Primary form of infection in humans comes from direct or indirect contact with sick animals or their products (Alterkruse et al., 1998). Some cases of human-to-human transmission have been reported (Tuon et al., 2017). The direct form of infection occurs when the person has been exposed to contact with aerosols, uterine secretions, placentas from sick animals, aborted fetuses, amniotic and birth fluids, or even laboratory samples (Davis & Elzer, 2002; Valderas M W & Roop M N, 2006). The bacteria can enter the human host via the oral route, inhalation of infectious particles, or through puncture wounds such as needlesticks (Kaufmann et al., 1980; Franco et al., 2007). Indirect infection occurs mainly through consumption of unpasteurized milk from infected cows, goats, sheep or camels, or cheese made from contaminated milk (Borba et al., 2013; Musa et al., 2008; Young, 1995).

1.3. *Brucella* survival in the environment

Freezing allows *Brucella* to survive almost indefinitely (Radostis et al., 2002). It can also survive freezing and thawing, which explains its viability in frozen semen and its potential transmission through artificial insemination (S. C. Olsen & Bricker, 2017). In addition, bacteria can remain viable in the environment and certain substances and materials for days to months, especially in cold and humid environmental conditions (Table 1). *Brucella* do not withstand high temperatures, and pasteurization can eliminate it from milk. The bacterium is also susceptible to the most common disinfectants (Fretin et al., 2005).

Table 1. *Brucella spp.* survival time in different materials

Environement	*Survival time*
Solid surfaces in sunlight	4-5 hours
Tap water	114 days
Soil and manure	80 days
Dust	15-40 days
Water at 37 °C and pH 7.5	less than 1 day
Water at 8 °C and pH 6.5	more than 57 days
Fetuses kept in the shade	6-8 months
Straw	29 days
Wet soil at room temperature	66 days
Dried soil at room temperature	4 days
Animal waste	
Sheared wool	110 days
Leather stained with cow excrement	21 days
Animal waste at room temperature	7 weeks
Food	
Milking fat	9 days
Fresh cheese	5 days
Milk at room temperature	2-4 days
Fluids, secretions and excretions	
Bovine urine	30 days
Fluids and secretions in summer	10-30 minutes
Vaginal discharge kept on ice	7 months
Bovine feces	1-100 days

Source: H. A. Castro et al., 2005**;** Corbel, 2006; Gibbs & Bercovich, 2011; Plommet et al., 1988

1.4. Bovine brucellosis

Bovine brucellosis is a chronic infectious disease with worldwide distribution caused by *B. abortus*. Abortion, epididymitis and vesiculitis, birth of weak calves, reduced milk production, infertility, and subfertility in cows and bulls are the essential characteristics of the disease (Corbel, 1997).

In the farm, the disease is spread by contact with secretions from infected animals. Cows also shed the bacteria through their milk and can infect humans (Chand & Chhabra, 2013).The disease has no treatment and its control is based on the use of vaccines: in calves between 3 and 8 months of age with the *B. abortus* strain 19 vaccine and in unvaccinated adult animals with the *B. abortus* strain RB51 vaccine (Poester et al., 2013 ; Samartino, 2002).

1.5. Brucellosis in humans

Four *Brucella* species are potentially dangerous for humans: *B. abortus, B. melitensis, B. suis, and B. canis* (Peng et al., 2020). Cattle are the primary source of contamination in humans, and the most frequent cases of contagion are produced by *B. abortus and B. melitensis*. Newly recognized marine mammal *Brucella* species have been shown to have zoonotic effects. Other species may also have clinical significance (Table 2). However, they are not frequent and are usually restricted to endemic areas (Franco et al., 2007b; Godfroid et al., 2011; Macías et al., 2019).

Table 2. *Brucella* species, hosts and zoonotic potential

Species	*Natural host*	*Zoonotic potential*	*Reference*
B. melitensis	Sheep, goats and camels	High	Bruce, S. D. 1887
B. abortus	Cattle, elk and bison	High	Bang, B. 1897
B suis	Pigs, hares, reindeer/caribou	High	Traum, J. 1914
B. inopinata	Unknown	Moderate	Scholz et al 2018; Eisenberg, T.
B canis	Dogs (domestic and wild)	Moderate	Carmichael, L. E., & Bruner, D.
B. ceti	Cetaceans (Whales and dolphins)	Moderate	Foster et al. 2007
B. pinnipedialis	Pinnipeds (Seals, sea lions)	Low	Foster et al. 2007

The infection in people occurs acutely or chronically with non-specific symptoms, since they will depend on the organ in which the pathogen is located (Galinska & Zagorski, 2013). However, flu manifestations and recurrent febrile are common conditions (Franc et al., 2018). Patients may experience the so-called undulant fever accompanied by chills, fatigue and muscle pain, malaise, arthralgia, myalgia and back pain, anorexia and prostration, which occur with greater or lesser frequency (Galinska & Zagorski, 2013). In some cases, infected people can develop a severe form of the disease, which includes endocarditis, arthritis, orchitis, epididymal-orchitis, and neurological disorders requiring prolonged periods of antibiotic treatment and lengthy convalescence (Pal et al., 2017). In the absence of specific treatment, the acute phase may evolve into a chronic phase with relapses, the development of persistent, localized infection, or a non-specific syndrome resembling "chronic fatigue syndrome." The clinical picture can persist for weeks or months (Laplume et al., 2013). Although brucellosis in humans is rarely fatal, it can be severely debilitating and disabling. Complications in pregnant women are usually sequelae of the disease (Franco et al., 2007; Franc et al., 2018).

1.6. Bovine brucellosis impact in animal production

B. abortus can cause losses in several species of productive interest (cattle, goats, sheep and pigs) (Galinska & Zagorski, 2013). The economic losses are considerable due to abortions, extension of days open or without pregnancy, and trade restrictions on both infected animals and products (S. Olsen & Tatum, 2010). In dairy production species, milk is contaminated for life, forcing the farmer to discard and slaughter females, with an enormous cost associated with efforts to eliminate the disease on the farm (Cárdenas, 2018a; Laplume et al., 2013; Nicoletti, 1980).

1.7. Brucellosis impact in public health

Human brucellosis is the most widespread zoonotic disease globally, with around 500,000 new cases per year (Godfroid et al., 2005, Eales et al., 2010). The presence of symptoms of brucellosis in affected people reduce their work capacity, and they are forced to incur medical care and treatment expenses (Corbel, 1997).

Brucellosis is considered an occupational disease since it affects a large proportion of people who have contact with infected animals, such as farmers, animal caretakers, veterinarians, slaughterhouse workers (Gwida et al., 2010 ; Tschopp et al., 2022) and even staff from the laboratory that can be accidentally infected (Stableforth, 1952). On the other hand, new evidence suggests that maternal infection in humans represents a significant risk factor for pregnancy, including an increased risk of spontaneous abortion during the first and second trimesters of pregnancy, preterm delivery, and vertical transmission to the fetus (Arenas-Gamboa et al., 2016).

Because *Brucella* can colonize different organs, the symptoms of the disease are usually non-specific, making diagnosis difficult and complicating the treatment (Sauret & Vilissova, 2002). As a result, the disease is not usually among the possible diagnostic repertoires of doctors, and sometimes the clinical picture is attributed to other pathologies (Cárdenas, 2018). In countries where diseases such as malaria or dengue are endemic, diagnoses based on clinical signs are often confused, and brucellosis patients end up being treated for vectorial diseases (Amexo et al., 2004; Araque-Villaquiran et al., 2021). Despite its importance, brucellosis can be considered as a neglected disease, whose interest increases only when outbreaks in humans are recorded (Welburn et al., 2015; Arenas-Gamboa et al., 2016).

2. Geographic distribution of bovine brucellosis

Bovine brucellosis is an enzootic disease in most parts of the world. However, there are areas considered free of the disease. According to the provisions of the WOAH, there may be free countries or zones with or without vaccination. For a country to be considered free without vaccination, it must meet a series of requirements including the following: to consider the disease notifiable throughout the country, not having recorded outbreaks in the last three years, surveillance through laboratory testing of herds, having established measures for the early detection of the disease and not applying the vaccine to cattle.

Australia, Canada, Israel, Japan, New Zealand and some European countries are considered free of the disease. In the United States of America, the absence of *B. abortus* in domestic herds has been recognized; however, the disease continues to be present in wild reservoirs, mainly in elks (Davis & Elzer, 2002 ; Kauffman et al., 2016). Bovine brucellosis remains enzootic in countries of the Middle East, the Mediterranean region, sub-Saharan Africa, China, India, Mongolia, Russia, and the Americas (except Canada and the United States of America) (Ponomarenko DG et al., 2020; Refai, 2002). The bovine brucellosis status, according to the reports from countries to WOAH for 2020, is shown in Figure 1.

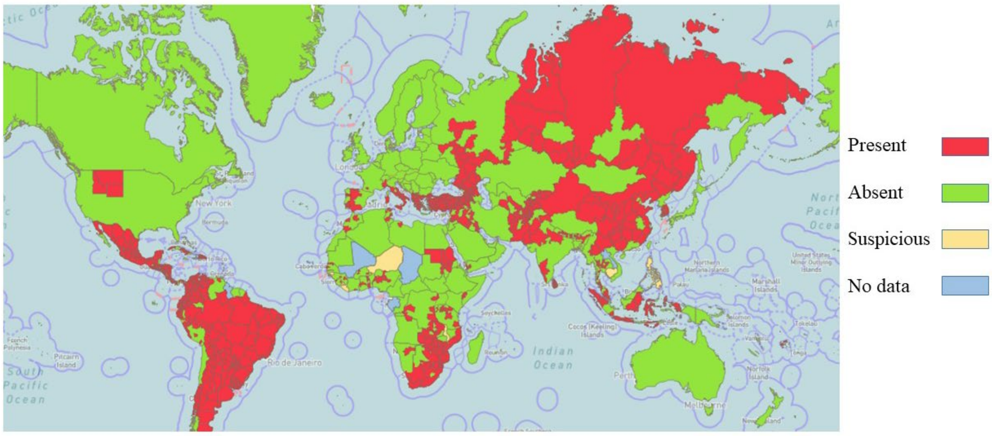

Figure 1. Bovine brucellosis status in the world for 2020 (Wahis-WOAH, 2023)

2.1. Situation in Latin America

In Latin America, at least four out of ten people live in areas where bovine brucellosis is endemic in animal reservoirs (Álvarez, 2001). According to the Wahis-WOAH information system, reports on the presence of bovine brucellosis in Latin America have increased since 2010 (Figure 2).

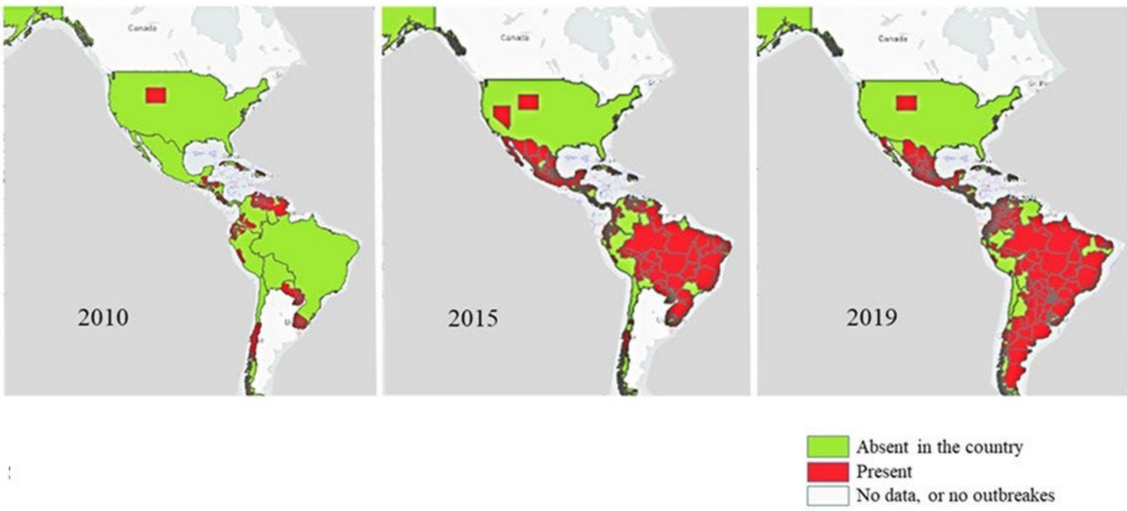

Figure 2. Bovine brucellosis situation in the Americas in 2010, 2015 and 2019 (Wahis-WOAH)

In Mexico, which has one of the largest bovine inventories with about 34 million cattle, *B. abortus* within-farm prevalence of 20% has been reported (Rojas Martínez et al., 2021). Between 2012 and 2017, approximately 2,000 cases of brucellosis were recorded (Lozano-López et al., 2022).

In Colombia, with a bovine inventory of more than 29 million cattle, between-farm prevalence values between 20% and 40% were recorded in a study (Motta-Delgado et al., 2018). Brucellosis in humans would be underrecorded.

Regarding Brazil, the largest country in South America with the largest bovine population in the world (159.3 million heads), bovine brucellosis is present in all states (between-farm prevalence from 2.52 to 41.5%) and affects cattle and buffaloes (D. Castro, 1982; Oliveira et al., 2019). The highest prevalence occurs in areas with the highest livestock density (Poester et al., 2002; Santos et al., 2013). Human brucellosis in Brazil is considered an occupational disease that mainly affects slaughterhouse personnel (Ramos et al., 2008).

In the case of Argentina, which has a bovine inventory of approximately 48 million heads of cattle, bovine brucellosis is an endemic disease (England et al., 2004; Samartino, 2002). Between 10 and 13% of the animals would be infected with an individual prevalence of 4-5% in cattle, and 50% in buffaloes. Between 1993 and 1995, 212 cases were diagnosed in humans of which 118 cases were detected in rural areas and were associated with contaminated goat cheese (Samartino, 2002; Wallach, 1995).

Bovine brucellosis has also been reported in Bolivia, Peru and Paraguay. In Chile and Uruguay the disease has been controlled and its prevalence has been reduced (SAG, 2023; Cárdenas, 2018).

3. Situation of bovine brucellosis in Ecuador

3.1. Characteristics of cattle farms

In Ecuador, as of 2018, there were 4,306,244 cattle recorded and distributed in the three regions of the country as follows: 52% in the Andean region called Sierra, 40% in the coast region and 9% in the Amazon region (INEC-ESPAC, 2019).

Around 205,833 farms raise cattle. Cattle farms of less than 10 ha are considered small farms, farms between 10 ha to 50 ha are considered medium farms, and those who have more than 50 ha are considered large farms. Most of the farms (92%) are small and medium farms (Haro Oñate, 2003). Most of the large farms are specialized in producing meat or milk. Small and medium farms develop dual-purpose cattle because they offer daily income from the sale of milk or cheese in addition to temporary income resulting from the sale of animals for slaughter or breeding (Torres et al., 2015).

At the national level, creole and crossbreeds predominate. However, in the coastal region, there is a significant percentage of zebuine breeds (Brahman). In contrast, in the Sierra region, the Holstein breed is more frequent (INEC, 2017).

3.2. Cattle trade in Ecuador

In Ecuador, cattle bought on farms tend to be moved from one market to another until the selling prize seems attractive for the trader. Cattle intended for slaughter may have been previously traded in four different markets. Although the small producer prefers to trade directly with other farmers in his region to ensure payment, one or more intermediaries (retailers) are involved in many cases (Zambrano LA, 2019). The retailer is responsible for moving the animals to markets or to slaughterhouses. However, in many cases, the retailer sells the animals to other retailers who are responsible for transporting the animals to other markets or to slaughterhouses (Ríos-Núñez & Benítez-Jiménez, 2015).

3.2.1. Control of cattle movements

To transfer animals, an animal mobilization guide is required, which is a mandatory official document issued by the control authority through a virtual system called SIFAE. This system was elaborated within the framework of the control and eradication program of foot and mouth disease (FMD) in Ecuador (Agrocalidad, 2011; MAG, 2023). The collected information includes the origin and destination of animals, the number of animals moved, age category, and history of vaccination against foot-and-mouth disease. The mobilization guide is reviewed at different checkpoints in all country provinces.

3.3. Situation of bovine brucellosis

In Ecuador, bovine brucellosis is widespread throughout the territory except in the insular province of Galapagos, which can be considered as free from the disease (Gioia et al., 2019). It has been estimated that bovine brucellosis causes annual losses of 5.5 million dollars in Ecuador due to abortions, reduced milk production, confiscation and slaughter of positive animals (Agrocalidad, 2008). In 1979, the first national serological survey of brucellosis was carried out that allowed to establish three epidemiological regions according to the percentage of animal prevalence per province as follows: 2% to 11% in the northern and central Andean provinces, 1% to 2% in the central Andean provinces and 4% to 10% in the coastal provinces (Agrocalidad, 2009).

Studies carried out in the three regions of the country have shown between-farm prevalence values ranging from 24 to 48% and from 1% to 9.73% at the animal level; (Díaz Albuja & Lamiña Juiña, 2013 ; Carbonero et al., 2018 ; Poulsen et al., 2014 ; Salguero Iza, 2014; Zambrano Aguayo & Pérez Ruano, 2015).

In 2018, the official veterinary services (Agrocalidad), with the support of international organisations, implemented an epidemiological survey to establish the prevalence of bovine brucellosis at the national level. Based on the bovine national census, a random sampling design was carried out, including females older than 24 months. A total of 290 farms and 3,752 females were sampled. An overall farm-level prevalence of 21.4% and an animal prevalence of 5.7% were found (Agrocalidad, 2019). Positive and negative farms are shown in figure 3.

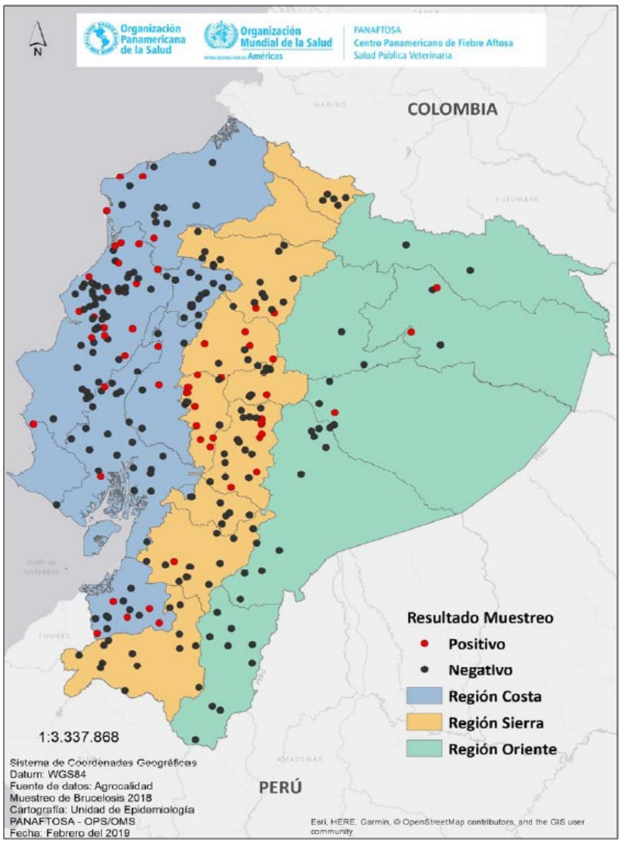

Figure 3. Spatial distribution of cattle farms with positive and negative results to *Brucella spp.*, in 2018 in Ecuador (Reproduced with permission of Agrocalidad)

Some studies have looked at prevalence levels in slaughterhouses. For example, in the study conducted in slaughterhouses in several cantons of Manabí province, a prevalence value of 1.06% in animals was established (Zambrano Aguayo & Pérez Ruano, 2015). Besides, 193 slaughterhouse workers were sampled in the same study, and a prevalence of 1.04% was found.

Cases of brucellosis in humans have been reported in several provinces by the Ministry of Public Health (MSP, 2021). The number of cases reported from 1990 to 2008 was 0.21 cases per 100,000 people. In a study conducted in humans a prevalence of 2% among people was related to cattle management (Ron-Román et al., 2014). Most human cases have been directly related to the presence of the disease in the bovine population (Aguilar, 2009; Ron-Román et al., 2014; Zambrano Aguayo & Pérez Ruano, 2015).

3.4. Risk factor studies in Ecuador

A *risk factor* is any environmental or endogenous characteristic that precedes the onset of the disease and is associated with disease deterioration (Grundy, 1973). A risk factor is causal when its presence contributes to explaining the occurrence of a disease and constitutes a marker when it only increases the probability of the effect occurring without its presence helping to explain the occurrence of the condition (Álvarez-Martínez & Pérez-Campos, 2004).

Some studies about bovine brucellosis risk factors have been conducted in some provinces of Ecuador on milk farms and mixed farms of medium- and large-sizes. The identified risk factors included: the age and time spent of an animal on the farm, the sex of animals (higher in cows), the type of production (higher in milk cattle), the lack of information on health status at the time of purchase, the absence of vaccination, the type of facilities and the introduction of cattle coming from livestock markets (Zambrano Aguayo et al. 2016; Maniato S & Vallecillo 2017; Carbonero et al. 2018 E; Maniato E. et al. 2022).

3.5. Official bovine brucellosis control plans

In 2008, the "National Brucellosis Control Plan" and the "Free Farm Certification Plan" were developed. A ministerial agreement included compensation for producers of USD 0.1 per litre of milk from free farms (Agrocalidad, 2016). However, given the non-compulsory nature of its implementation and the high costs of the disease diagnosis, the small and medium-sized farmers did not get involved in the program, (Paucar Quishpe, 2019; Zambrano Aguayo & Pérez Ruano, 2016). A second plan was established in 2018 that included control strategies such as the compulsory and massive vaccination of bovine females under 15 months of age, diagnosis and elimination of reactors but has not yet been implemented (Agrocalidad, 2019).

According to the official manuals, the disease can be classified as a suspected case when a clinical picture of late abortion, retained placenta or infertility occurs in cows. All abortions in cattle should be considered suspected cases of brucellosis and should be investigated.

The accepted laboratory tests are those recommended by the WAHO in the diagnostic manual for terrestrial animals. Currently, the test of choice is the Rose Bengal test. It is routinely used at the national and local levels to diagnose animal and human brucellosis and must always be confirmed with a bacteriological or serological test. The most used serological confirmation test is the competitive ELISA.

Vaccinal strains 19 and RB-51 are used in Ecuador according to national regulations. Strain 19 should be applied in female calves between 3 and 6 months of age as a unique subcutaneous dose. However, the farmer is warned that some animals can develop long-term antibodies titles. RB51 vaccine is recommended in females of 4 months and older and would have the advantage of not interfering with the results of diagnostic tests (Agrocalidad, 2016).

4. Social network analysis methodology

4.1. Background

The concept of social network was first presented in 1954 in an article on anthropology, which emphasized the social aspect of the relationships and connections between the nodes of a network (Stattner & Vidot, 2011). The Social Network Analysis (SNA) methodology provides tools to understand the deep context of disease dynamics related to biological, sociological, and economic, among others (Brandes et al., 2013). The social network approach has been rapidly integrated into epidemiology, as a network allows representing the dynamics of contacts that are known to influence the way a disease spreads. SNA is also used to monitor the evolution of the disease, design interventions to stop its spread or prevent contagion, and to estimate the impact of control strategies (Christakis & Fowler, 2009; Neaigus, 1998). Similarly, the structure and nature of the network may be related to the evolution and speed of the spread of disease (A. M. El-Sayed et al., 2012).

4.2. Graph theory

The SNA has its origin in the graph theory. This theory took place in 1736, in an article published by Leonhard Euler, who proposed the well-known problem of the Königsberg bridges. The problem stated that in the city of Königsberg, there were seven bridges connecting different regions. As a result, the city's inhabitants took Sunday walks, trying to find a way to walk across the city, crossing each bridge only once and returning to the starting point. To solve this problem, Euler represented the four zones as four points and the bridges as links connecting the points to form a graph (Douglas B. West, 2000). A graph then became a structure composed of nodes connected through links. This gave way to a series of mathematical applications in which graphs could represent relationships between various entities (Mesbahi & Egerstedt, 2010).

4.3. Network definition

In general terms, networks are graphs that allow the modelling of complex structures that can be analyzed in their shape, size, structure and connectivity (Wasserman et al., 1994). The fundamental elements that define a network are the entities represented by nodes and their interactions as links (Albert-László Barabási, 2016; Newman, 2010).

The study of social networks focuses on analyzing the interactions of a group of entities that make up the network. Individuals, represented by nodes, are linked to each other based on their interactions represented by links. These links can be directed or undirected. In undirected graphs the direction of the link of a pair of nodes A and B is not taken into account, whereas in a directed graph A and B can be connected in three ways: either by a 'single link' (A→B or A←B) or by a double link (A ↔B) (Palla et al., 2007).

4.4. Network indicators

There are several types of indicators that are used to describe a network, including indicators of centrality, cohesiveness and connectivity.

Centrality indicators include *degree*, *betweenness* and *closeness* (Martínez-López et al., 2009). *Degree* is the number of connections that a node has with other nodes. If a network is directed, there will be links going out and links coming in from the nodes. Therefore, it is said that there are degrees of exit (*out-degrees*) and entry (*in-degrees*) depending on the disposition of the incoming and outgoing links of the nodes (Barabási, 2003)*,* *Betweenness* is an indicator that measures the frequency with which a node appears on the shortest paths between other nodes (Freeman, 1978). *Closeness* defines a node as central if it is closer to other nodes on average. It facilitates the identification of nodes that are important for disease spread in the network (J. Enright & Kao, 2018)

The *cohesiveness indicators* assess the level of connectedness of the network as a whole and help to identify weaknesses in the structure of the network (Martínez-López et al., 2009). They include *density*, *average path length* and *clustering coefficient.* From the epidemiological perspective, this indicators facilitates the identification of nodes that are important for disease spread in the network (J. Enright & Kao, 2018). The *density* is the number of relationships existing in the network between all possible relationships (VanderWaal et al., 2016). The *average path length* is linked to the speed of the spread of a disease as it corresponds to the average of the shortest number of nodes that need to be traversed to reach each of the other vertices of the network, also termed *geodesics* (Shirley & Rushton, 2005). The *clustering coefficient* of a node in a graph quantifies how clustered, or interconnected, each node is with its neighbours. It also represents the proportion of neighbours who are neighbours of each other (Watts & Strogatz, 1998). A high level of *clustering coefficient* means that most infections occur locally (Keeling & Eames, 2005).

Other indicators allow measuring how the nodes are connected to each other. *Assortativity* is the tendency of nodes to connect with nodes of similar or different degrees through links of the same type (Newman, 2002). *Reciprocity* is related with the proportion of nodes that connect with others in the opposite direction (Wasserman et al., 1994).

The *components* are subsets of more extensive networks in which all pairs nodes are directly or indirectly linked. They can be classified into *strong* and *weak components*. In *strong* components, direct routes (i.e. taking into account link directions) connect the nodes to each other. In *weak components*, the nodes are connected by at least one path through the network and are mutually reachable, in this case without considering the link direction (Dubé C. et al., 2011; Robinson S.E & Chistley R.M, 2007).

Communities correspond to network sub-groups, in which specific nodes can be linked more closely because they share properties or act similarly within that network (Christakis & Fowler, 2009; Newman, 2006). In the case of veterinary epidemiology, identifying *communities* makes it easier to determine the geographical areas where the animal exchange takes place more intensively, and through this to plan a risk-oriented surveillance system or the implementation of regional health programs (Grisi-Filho & Amaku, 2015). On the other hand, knowledge of the existence of exchange *communities* could be helpful for a more rigorous application of control plans such as vaccination and surveillance (Christley et al., 2005; Natale et al., 2009).

4.5. Network topology

There are different types of networks. A *random network* is a type of network in which the nodes are connected randomly. Random networks are characterized by short paths and small clustering coefficients (Martínez-López et al., 2009). A *regular network* has as a main characteristic that all the nodes have the same degree. A regular lattice's characteristic is that a node's neighbourhood corresponds to the adjacent nodes. Also, the average geodesic distance is high since the connections are mainly local (Álvarez-Cabrera, CE et al., 2015). A *small-world* network is characterised by high clustering coefficient and low average path length. This type of network represents a situation in which most nodes have neighbours within walking distance, but some few would have neighbours far away. This means the epidemic would be localized in a neighbourhood, and its spreading speed would be slow. However, the random connections of some individuals significantly impact the dynamics of the epidemic since such connections spread the epidemic to nodes far away. Infectious diseases will spread much easier and faster in a *small world* due to the few shortcuts needed in the small world scheme (Watts & Strogatz, 1998).

A *free-scale* network is a type of complex system in which a small number of nodes have a large number of connections to many other nodes, while most nodes have only a few. In this network, the degree distribution follow a power law distribution.

4.6. Percolation analysis

Percolation is a process that describes the behavior of a network when nodes or links are removed to achieve fragmentation (Sahini & Sahimi, 2003). The percolation analysis allows establishing at what moment a strong component is disintegrated through the elimination of central nodes (Schwartz et al., 2002). In the context of a pathogen spread, it has been shown that removing these entities in disassortative directed networks would have an effect in controlling disease spread (Lentz et al., 2016). For example, in livestock movement networks, the removal of entities (farms, parishes, markets) is achieved in a direct way by banning livestock movements or in an indirect way by vaccination (Palisson, 2017).

4.7. Application of the SNA methodology in veterinary epidemiology

The increasing availability of data and the high coverage of information in recent years has allowed many countries to quantify the relationships between animal movements and disease spread, facilitating new studies ranging from demographic structure research to pathogen dynamics (Kao et al., 2006; Natale et al., 2009). Some studies have focused on identifying network topology and structure as these characteristics can reveal how fast an agent can spread through the entire system. SNA can also help to understand the potential spread of an agent during the silent spread phase, which corresponds to the period between the introduction of an infectious agent into a population and its first detection (Dubé C. et al., 2011). The use of SNA in epidemiology has also been extended to the study of the dynamics of infectious agents (Tuckwell et al., 1998), the development of epidemiological models (Keeling & Eames, 2005), epidemiological surveillance or the design of disease control strategies, among other applications (Balcan et al., 2009 ; Christley et al., 2005).

4.7.1. SNA and livestock trade

Mechanisms related to animal trade can contribute to the spread of a disease (Assenga et al., 2015; Dubé et al., 2009; Robinson S.E & Chistley R.M, 2007). The movement of animals can cause diseases to jump between locations separated by large distances and may reach areas where the disease was previously absent (Gilbert et al., 2005 ; Robinson S.E & Chistley R.M, 2007). The 2001 epidemic of foot-and-mouth disease in Great Britain, which originated in a sheep farm and then spread rapidly through farms and markets across the United Kingdom territory, highlighted the relationship between livestock movement and the risk of disease spread throughout the country (Gibbens et al., 2001).

Many countries have established regulations for farmers to report livestock movements to authorities. Livestock movement records have been used as sources of information to which network analysis tools have been applied to successfully identify system properties, highlight vulnerabilities to transmission, and inform targeted surveillance and control (Chaters et al., 2019).

The integration of cattle trade data in epidemiological studies and the use of SNA have allowed studying the relationship between animal movements and the spread of infectious diseases of cattle. This was the case for slow-spreading diseases such as bovine tuberculosis in Great Britain (Green et al., 2008) and France (Bouchez-Zacria et al., 2018; Palisson et al., 2017), or paratuberculosis in dairy cattle in Italy (Rossi et al., 2017). Fast-spreading diseases have also been addressed using SNA, for instance the relationship between avian influenza spread and the movements of backyard birds in Thailand (Poolkhet et al., 2013) the potential spread of FMD in Peru (Martínez-López et al., 2009).

SNA tools have been used to describe and analyze the cattle movement networks in Argentina (Aznar et al., 2011), Uruguay (VanderWaal et al., 2016) and Brazil (Júnior et al., 2017). Those works have provided information to strengthen epidemiological surveillance in those countries.

5. Qualitative methodology in social studies

Qualitative research is a scientific method based on the collection of non-numerical data through observation. It consists in the use of various means to collect the complete discourses of the participating subjects, and then proceed to their interpretation, analyzing the meaning of their criteria according to specific social structures. Data collection techniques include interviews, focus groups and participant observation, among others. Qualitative research is mainly used in the social sciences, such as anthropology and sociology. However, it has now begun to be used more frequently in other fields (Wagenaar & Babbie, 2008). Qualitative approaches are useful when the central question of interest is "why" rather than "how much." (Waters & Gallegos, C, 2014). They are particularly suited to public health research because people make health decisions and their actions are based on complex factors that cannot necessarily be accurately measured by quantitative methods (Ulin et al. 2005).

Qualitative research works with people, situations, observations, stories, behaviors, organizational functioning, and social movements, among others (Miles and Huberman, 1994). Qualitative research techniques basically use words, not numbers, to communicate findings (C. I. Ivankovich-Guillén & Araya-Quesada, 2011). There are many qualitative research techniques, which are applied according to the research question and needs. These include ethnographic studies, individual or group interviews, and participatory observation strategies such as focus groups, among others (Miles M. & Huberman M., 1994). These techniques allow the collection of information from direct sources and allow a deep inquiry into knowledge because they are developed through the free expression and self-explanation of the participants about their criteria, experiences, perceptions and beliefs (C. Ivankovich-Guillén & Araya-Quesada, 2011). One of the advantages of this methodology is that interviewees can answer essential questions that are often not achieved with traditional research techniques (Huston & Rowan, 1998).

Unlike quantitative research, in qualitative research, it is possible to work with small groups, which allows a topic to be explored in greater depth (Carlsen & Glenton, 2011). The information-gathering process is carried out during the interview, and information collection depends on the “redundancy” of the information or “theoretical saturation. Theoretical saturation corresponds to the "process of conducting interviews sequentially until all concepts are repeated several times with no new concepts or themes emerging." (Cleary et al., 2014). Theoretical saturation describes how new contributions to findings have ceased to be obtained from observations (Trotter, 2012) and implies that the required data has been obtained (Hernández Sampieri, 2010).

In qualitative research, there are several types of sampling, including purposeful sampling. In this type of sampling, the participating groups are selected according to the interest and objectives of the research.

5.1. Grounded theory, coding and data reduction

Grounded theory is a rigorous qualitative research methodology that allows to build a theory that explains the results obtained in the qualitative research process (Glaser & Strauss, 2010). It allows the theorization of possible explanations to the observed facts, hence its name. A study based on Grounded Theory begins with a general question, for example: under what specific conditions does this event occur, how is it manifested, by whom, when, where, how, and with what consequences (Cuesta-Benjumea, 2006; Glaser & Strauss, 2010).

The discourse of the study participants allows the recording of concepts (data) that can form patterns and sub-patterns (Glaser, 2002). The identification of patterns is made by capturing phrases and words that fit into a category that may be pre-established. These pre-established categories, called *a priori* categories, are usually broad themes or issues established prior to the interviews or observations (McMillan & Schumacher, 1984). The categories and their relationships between them are analyzed and compared until a theory is constructed based on these data (Glaser & Strauss, 2010). The theoretical concepts formulated in the data interpretation are constructed in the process of analysis itself. In this sense, in studies that aim to generate a theory, the theory will emerge and develop during data collection and analysis (Ulin et al., 2006).

Data reduction refers to the process of selecting, focusing, simplifying and transforming the data that appear in written-up field notes or transcriptions through coding, teasing out themes, making clusters or partitions or writing memos. *Coding* is a qualitative data analysis strategy in which some aspect of the data is assigned a descriptive label that allows the researcher to identify related content across the data (Deterding, N.M & Waters, M. C., 2018).

5.2. Focus groups

A focus group or focus interview is commonly defined as collecting research data through a moderated group discussion based on participants' perceptions and experience of a topic decided by the researcher (Carlsen & Glenton, 2011). The data sources are the participants' judgments and interventions collected and categorized.

Focus groups have advantages for health and medical researchers: they do not discriminate against people who cannot read or write and can encourage the participation of people who are reluctant to be interviewed on their own or who feel they have nothing to say (Kitzinger, 1995). In addition, working in groups facilitates the discussion and activates participants to comment and give their opinions even on those topics that are considered taboo, thus generating a wealth of testimonies.

According to the research objective, the interview guide and the logistics for its achievement are determined, including the participants' choice, the sessions' scheduling, strategies for approaching them, and the call, among others. The focus group promotes discussion among the research participants based on open questions formulated by a facilitator (Huston & Rowan, 1998). The information from the participants' interventions allows for obtaining coded, grouped, categorized and interpreted data to obtain interpretable results (Ulin et al., 2006).

Focus groups differ from group interviews in that the emphasis is on the interaction between participants rather than between the moderator or researcher and the participants.

5.3. Applications of qualitative research in public health

Initially, studies with a qualitative approach were mainly developed in psychology, psychiatry and psychoepidemiology (Clement et al., 2015; Zamudio-Rodríguez et al., 2021). However, qualitative research has become a popular form of current public health research because it allows researchers to analyze health-related social phenomena (Malterud, 2002) and help to understand their implications for health-related decision-making (Cockerham & Scambler, 2021).

Qualitative techniques in different health research settings have been applied in several countries. For example, in Brazil, they have been used to evaluate the impact of health promotion strategies among the population (Lervolino & Pelicioni, 2001). In Colombia, they have been used to establish the population's perception of risk associated with factors such as knowledge of dengue transmission and the population's attitude towards arbovirosis control (Benítez-Díaz et al., 2020). Some studies have evaluated the risk associated with the knowledge and attitudes of the population about social problems and chronic or infectious diseases. For example, in Ecuador, ageing-related health has been studied qualitatively by focus groups in people from several indigenous communities (Waters & Gallegos, C, 2014). In Puerto Rico, it has been helpful in the identification of beliefs and community practices related to dengue fever by analyzing differences in attitudes towards the disease and its prevention according to the sex of the respondents and their history of having suffered from this disease, using the focus group technique (Perez-Guerra et al., 2009).

5.4. Applications of qualitative research in veterinary epidemiology

Qualitative methodology has been little used in the veterinary field. However, with the increasing complexity of the veterinary field, qualitative research tends to become relevant in the search for answers to new and equally complex research questions (May, 2018; King et al., 2021).

Studies have been mainly directed to the study of zoonoses, such as the one conducted in China to identify the main factors of population behaviour associated with the threat of new zoonoses (Li et al., 2020) or the study for the characterization of the social determinants of rickettsiosis in a region of Colombia (Peña-Ríos et al., 2019). Research under a qualitative approach has been helpful in studies on backyard and nomadic producers to learn about pig-raising practices linked to the presence of diseases (Nahar et al., 2012) or the interaction of people with their poultry and the zoonotic transmission of avian influenza in Bangladesh (Sultana et al., 2012).

Some work has been developed under the One Health framework, such as prioritising zoonotic diseases to be addressed in Kenya through focus groups composed of veterinarians, wildlife biologists and government doctors (Munyua et al., 2016). Under the same framework, the grounded theory approach was applied to evaluate brucellosis control in Kazakhstan (Charypkhan & Rüegg, 2022), or in Sierra Leone to understand the implications of hunting activities of women and children on zoonosis control (Bonwitt et al., 2017).

Some studies have applied the methodology combined with quantitative techniques, such as the one conducted in Argentina on evaluating zoonoses risk perception and population behaviors in adults (Villacé et al., 2018).

In Ecuador, the focus interview technique was used to study the determinants of antibiotics use in small-scale food animal producers (Butzin-Dozier et al., 2020).

6. Problems and main lines of research

Cattle production in Ecuador is the main livestock activity and a fundamental pillar of the Ecuadorian agricultural sector. Livestock development has favored the production of foodstuffs such as meat and milk, which are fundamental for the population's food security, as well as the supply of raw materials for the production of other consumer goods. Livestock production also involves peasant labor, which contributes to the dynamism of the rural economy. The enzootic presence of bovine brucellosis in Ecuador not only causes losses in this livestock sector, it is also a public health threat.

Although there are studies that have focused on establishing the prevalence of bovine brucellosis in several areas of the country, aspects that may be facilitating its spread or hindering the efficient implementation of a control program, such as cattle movements and the way farmers deal with the disease, have not yet been investigated. In this context, the main objectives of this PhD thesis were to study the potential implication of the cattle trade network in the spread of bovine brucellosis in Ecuador and the role played by small and medium scale farmers in the maintenance of the disease.

Trade of animals and products in Ecuador takes place throughout the national territory through a road network that connects the 23 provinces of the country and allows the most distant places to be reached in the same day. So far, in Ecuador, the movement of cattle is only authorized with a vaccination certificate for FMD. Animal trade can contribute to the spread of infectious diseases such as bovine brucellosis, as it facilitates the movement of infected animals from one area to another. In this regard, three questions arise: i) is it possible to structure a network that represents the cattle trade in Ecuador and allows to understand its main characteristics, such as size, structure and connectivity? ii) could a network representing the cow trade help to understand the bovine brucellosis spread? iii) could a selective elimination of the nodes of those networks help to identify the most appropriate way to control the spread of a disease?

The cattle sector in Ecuador is composed of different productive systems that differ in their productive specialty, size and use of technology, and possibly the level of information and the level of awareness of producers regarding bovine brucellosis. Of the total number of registered farms in Ecuador, about 76% correspond to units with less than 10 cattle (INEC 2008). However, almost all of the studies concerning bovine brucellosis have been carried out on large farms. Very little is known about the role played by small and medium cattle farmers in the maintenance and dissemination of bovine brucellosis in Ecuador, mainly in some provinces where very few studies have been conducted. From this perspective, it is worth asking: i) what is the prevalence of brucellosis in the farms belonging to those categories of farmers? ii) what is the level of knowledge that those farmers have about the disease? iii) are there management practices or animal products consumed or sold in those farms that may constitute a threat to public health?

To go further about the social issues related with the control of bovine brucellosis, we sought to address the questions on the level of knowledge, awareness and risk perception of bovine brucellosis among small and medium farmers and to collect the opinions of animal health professionals on the control of the disease.

6.1. Scientific strategy

The research strategy to answer these questions is based on three axes described in the three chapters of this thesis.

A national program for the eradication of FMD in Ecuador was launched in 2010. It included the implementation of a national cattle movement database. Taking into account that the spread of bovine brucellosis between farms is mainly related to the commercial movement of cattle, we used the network methodology to analyze the cattle movements recorded in the database of cattle movements taking into account the two last years available at the smallest geographical scale. We considered pertinent to build two types of network to represent a fast-spreading disease such as FMD, for which the cattle movement database was originally created, and a slow spreading disease such as bovine brucellosis. The first chapter corresponds to the analysis of those two networks.

The second chapter corresponds to a study of the prevalence and risk factors of bovine brucellosis in small and medium-scale cattle farms conducted in a zone of Esmeraldas province, a tropical region of Ecuador. High-risk practices for human health were also identified.

The third chapter corresponds to a qualitative research study conducted using the focus group methodology. We sought to establish the level of knowledge, awareness, and risk perception of bovine brucellosis in a focus group of small-scale farmers and in a focus group of middle-scale farmers. Two focus group of veterinarians were also gathered to address the perception of professionals involved in animal health about the brucellosis situation in the field and the factors that could be hindering the implementation of the brucellosis control program in Ecuador.

Chapter 1. Network analysis of cattle movements in Ecuador

1. Introduction

For most infectious diseases of livestock, the infectious agent can be transmitted between farms through local transmission routes such as direct contact between animals or through insects, rodents, wind, or alternative hosts (Gilchrist et al., 2007). However, another form of disease propagation can occur between distant farms due to the transport of animals or infected material, causing the diseases to jump between places separated by large areas where the disease is absent and potentially reaching and spreading in remote places (Fèvre et al., 2006; Gilbert et al., 2005; Green et al., 2008).

In the frame of the FMD control in Ecuador, an FMD information system was setup to ensure that all moved cattle were vaccinated against FMD. The intense vaccination work carried out is evidenced by the absence of the disease since 2011 and the recognition of Ecuador as FMD free with vaccination by the WHOA. The information on cattle movements has been recorded in a database and the control of FMD vaccination of traded cattle is still enforced.

Considering that the use of network analysis have allowed studying the relationship between animal movements and the propagation of pathogens that can be fast-spreading pathogens, such as the FMD virus, or slow-spreading pathogens, such as *B. abortus*, the purpose of this work was to build two networks of cattle movements in Ecuador in order to compare: i) the potential spread of a serotype/topotype of FMD virus not included in the vaccines currently in use in a network including all cattle movements, and ii) the spread of bovine brucellosis through a cow trade network.

2. Materials and methods

2.1. Data processing

The Ecuadorian veterinary services developed two national databases in the context of the FMD control program established in 2010. One database contains information on the cattle inventory by farm collected during the FMD vaccination campaigns carried out every six months throughout the country. This database comprises information on farm location specifying three administrative units (from the smallest to the largest: parish, canton, province), cattle farm type (beef, dairy, mixed) and number of cattle by category (calf, heifer, cow, bull). For this study, we used data for January 2017 and January 2018 to create farm and cattle density maps. The second database gathers all information on cattle movements including the identification numbers of origin and destination holdings, date of movement, geographical origin and destination specifying the three administrative units aforementioned, type of holding at origin (farm or market) type of holding at destination (farm, market, slaughterhouse) and number and category of moved cattle (cows, bulls, steers, calves). Data for movements in 2017 and 2018 were extracted to construct the movement networks.

Cleaning of the dataset from the movement database was performed to eliminate duplicated data and to homogenize administrative unit names. For this last operation, we used names approved by the Ecuadorian Institute of Statistics and Census of Ecuador (INEC). As we found inconsistencies on the use of farm identification codes, we decided to aggregate data at the parish level to conduct the analysis. Farm and cattle numbers recorded in the inventory database were aggregated by parish taking the farm type into account. All the cleaning and data processing were performed using the R 4.0.3 software (R Core Team, 2020). The resulting dataset was imported into QGIS version 3.10 (Sutton T. 2004) to create farm and cattle density maps. Map layers were obtained from the INEC (http://www.geoportaligm.gob.ec/) and the location of markets was provided by the Ecuadorian veterinary services (Agrocalidad).

2.2. Network construction

In this study, the trade of cattle was represented by a network with parishes as nodes and cattle movements between parishes as links. The network was directed because cattle movements occur in just one direction, from nodes at origin to nodes at destination. In previous studies, the possible spread of diseases through fomites had been considered involving slaughterhouses, for example through a network of truck movements (Salines et al., 2017). As in our study only the cattle movements were taken as a potential source of disease spread, movements to the slaughterhouses were not included.

To consider disease spread characteristics within the network according to animal category, two distinct networks were constructed: a network including all animal categories and a network with only cows. The “all animal category network” (termed below “global network”) would account for a serotype or topotype not covered by the current FMD vaccine in use in Ecuador that could be transmitted between farms by the trade of cattle, whatever the age or sex of the traded animals. The “only cow network” (termed below “cow network”) would account for diseases that are mainly transmitted between farms by the trade of cows, like bovine brucellosis. These two networks were built by year (2017, 2018) and by month (24 months) yielding a total of 52 networks.

2.3. Network description

Several indicators were calculated for each network: the size of the network (number of nodes) and the number of links, the density (total number of links divided by the number of possible connections), the average degree (the degree is the number of parishes that a parish is connected to through cattle movements), the betweenness (frequency with which a node appears on the shortest path between other pair of nodes), the average path length (average number of links along the shortest paths for all possible pairs of network nodes), the diameter (the most extensive shortest path among all the shortest paths in the network), the clustering coefficient measures the extent to which neighbors of a node are also neighbors of each other, the reciprocity (the probability of nodes of being mutually linked) and the assortativity (the tendency of nodes to connect to similar nodes in terms of degree).

The Jaccard index (JI), which allows the measurement of the similarity between two data sets, was used to compare the nodes and links included in each network from month to month and from year to year. It takes values between 0 (no elements in common) and 1 (the samples are identical). A function to estimate the JI was created using the R 4.0.3 software (R Core Team, 2020).

The number and size of the strongly (SC) and weakly (WC) connected components were determined for both the global and cow networks at the annual scale. The components are sub-networks where all nodes are connected. They can be interpreted as areas of a network where connectivity is exceptionally high. In SC, all nodes are mutually accessible by directed links, while in WC, all nodes are linked without taking into account the direction of the links (Robinson S.E & Chistley R.M, 2007). Identifying the largest SC or WC of a network can give an estimation of the maximal size of an epidemic in the case of a disease introduction (Dubé et al., 2008). The closeness centrality (average length of the shortest path between a node and all other nodes) was calculated in the largest SC as it should be computed in connected networks.

Likewise, the six largest communities for both the global and cow networks at the annual scale were identified using the Walktrap algorithm and represented on maps. Communities are more densely connected groups within a network with nodes that share common elements (Newman, 2006). In our case, they corresponded to the parishes that carry out the most significant commercial exchanges.

A network can have scale-free or small-world properties. The scale-free networks are characterized by the presence of hubs, which are responsible for several striking properties for propagation of infections (Meloni et al., 2009). A small-world network represents a situation where most nodes have close neighbors, but a few nodes have very distant neighbors (Watts & Strogatz, 1998). To detect the presence of scale-free and small-world properties in our networks, we plotted their degree distributions on a logarithmic scale, and generated random networks having the same numbers of nodes and links, to compare their clustering coefficient and average path length values with those of our networks. Networks analyses were performed using the Igraph package for R 4.0.3. (Gábor Csárdi & Tamas Nepusz, 2005).

2.4. Percolation analysis

To evaluate the efficacy of targeted control measures to limit disease spread through a network, a percolation analysis was conducted at the annual scale. The term percolation has been borrowed from the field of physics; in the context of social networks, a percolation analysis is conducted to study the behavior of a graph following the removal of its edges or vertices (Essam, 1980). Percolation analysis allows assessing the most efficient way to disintegrate the largest strong component viewed as an estimate of the maximal size of an epidemic spreading on the network. In our study, two strategies were implemented: *i*) removing all the parishes where markets were located, and *ii*) removing nodes with the highest betweenness values iteratively until a largest strong component threshold size representing less than 10% of the total nodes was attained. A specific program was developed to conduct the percolation analysis using the R 4.0.3 software (R Core Team, 2020).

3. Results

3.1. Cattle inventory dataset description

The number of cattle farms recorded for 2017 and 2018 are shown in Table 3. Around 70% of the farms were dedicated to mixed production of meat and milk, 17% to milk production, and 13% to meat production. In 2017, the bovine population was slightly higher than in 2018 and the proportion of animals distributed in the different categories of farms followed those of cattle farms.

Table 3. Number of cattle and cattle farms recorded in Ecuador in 2017 and 2018

	Cattle farms (% total)		*Number of cattle (% total)*	
Category	*2017*	*2018*	*2017*	*2018*
Dairy	46,645 (17 %)	45,177 (17%)	620,894 (15%)	590,351 (14%)
Beef	33,604 (13%)	33,848 (13%)	546,682 (13%)	591,439 (14%)
Mixed	190,912 (70%)	187,507 (70%)	3,107,915 (72%)	2,994,836 (72%)
Total	271,161	266,532	4,315,229	4,176,626

The location of the 63 animal markets recorded in the country is shown in Figure 4.

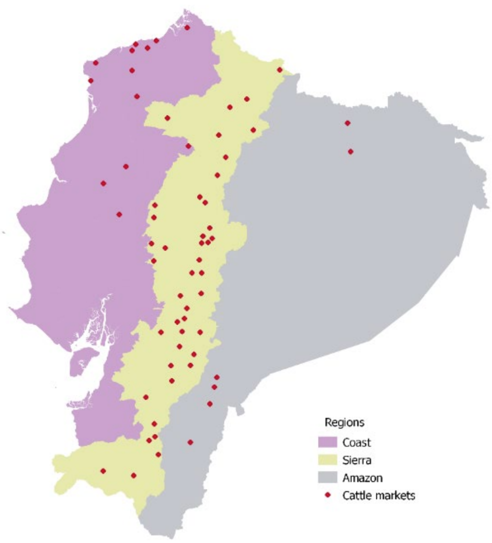

Figure 4. Ecuadorian geographic regions and location for the 63 animal markets present in 2017 and 2018

The cattle farm density was higher in the Sierra region, with some parishes recording up to 178 farms per km² (Figure 5a). These high densities corresponded mainly to small holdings. Cattle farm density differed per category with beef and mixed farms mainly concentrated in the Coast and Sierra regions (Figures 5b and 5d), while dairy farm density was higher in the Sierra region (up to 64 farms/km² in 2017) (Figure 5c). The highest farm density was found for mixed farms in a parish located in the center of the Sierra region (109, 3 farms/km² in 2017). Farm density was lower in the Amazon region but in some parishes beef and dairy farms were highly concentrated.

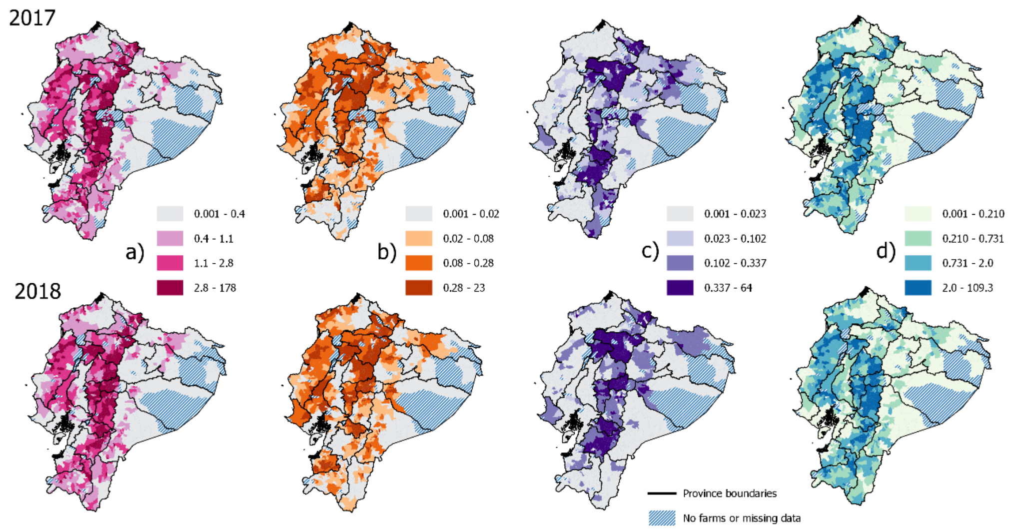

Figure 5. Density of all cattle farms (a), beef farms (b), dairy farms (c), mixed farms (d) per km2 in Ecuadorian parishes in 2017 and 2018

Density of cattle had a similar geographical trend as farm density for both years (Figure 6). The highest density value was recorded for cattle from mixed farms in a parish of the Sierra region in 2017 (652 cattle/ km²).

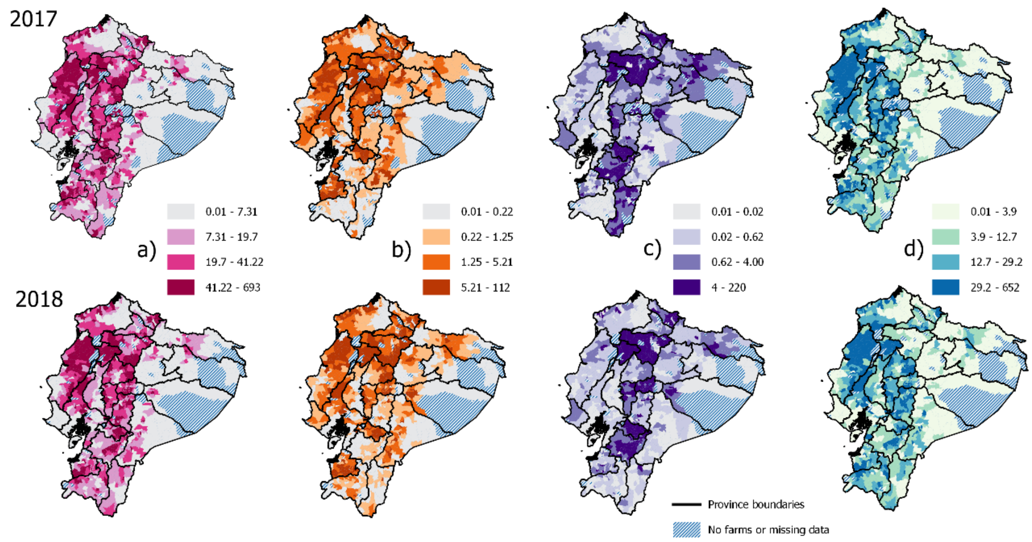

Figure 6. Bovine density: all cattle farms (a), beef farms (b), dairy farms (c), mixed farms (d) per km2 in Ecuadorian parishes in 2017 and 2018

3.2. Cattle trade dataset description

The number of cattle movements and cattle moved were close for both years (Table 4). The number of cow movements represented 46% of those for all cattle (441,453 out of 952,972 in 2017 and 441,451 out of 962,890 in 2018). Seventy percent of the cattle movements originated from farms for both years (675,652 out of 952,972 in 2017 and 674,668 out of 962,890 in 2018), of these approximately 15% (104,622 in 2017 and 106,660 in 2018) concerned movements to farms, 53% to markets (353,348 in 2017 and 367,955 in 2018) and 32% to slaughterhouses (217,682 in 2017 and 200,053 in 2018). All these proportions were similar for cow movements. The vast majority of movements were carried out between parishes either for all cattle or for cows only (77% in 2017 and 78% in 2017 in both cases). Cattle were moved in small groups of four animals on average (3,855,593 animals moved in 952,972 groups in 2017, and 3,814,917 animals moved in 962,890 groups in 2018), the group size being however larger for the between farm movements: 6-7 animals on average (725,026 animals moved in 104,622 groups in 2017, and 676,415 animals moved in 106,660 in 2018). Similar values were obtained for the movements of cows.

Table 4. Summary of cattle trade movements within and between parishes for all cattle (including cows) and only cows in Ecuador in 2017 and 2018

			Number of movements (% within parish)				Number of moved cattle (% within parish)			
Unit	Year	Destination / Origin	Farm	Market	Slaughterhouse	Total	Farm	Market	Slaughterhouse	Total
All cattle	2017	Farm	104,622	353,348	217,682	675,652	725,026	1,188,223		2,632,332
			(30%)	(19%)	(32%)	(25%)	(26%)	(16%)	719,083 (18%)	(19%)
		Market	190,843	23,665	62,812	277,320	794,797	82,750		1,223,261
			(17%)	(18%)	(24%)	(18%)	(15%)	(15%)	345,714 (19%)	(16%)
		Total	295,465	377,013	280,494	952,972	1,519,823	1,270,973		3,855,593
			(22%)	(19%)	30%)	(23%)	(21%)	(16%)	1,064,797 (18%)	(19%)
	2018	Farm	106,660	367,955	200,053	674,668	676,415	1,202,638		2,541,140
			(28%)	(20%)	(29%)	(24%)	(25%)	(17%)	662,087 (16%)	(19%)
		Market	203,470	20,425	64,327	288,222	873,933	73,332		1,273,777
			(17%)	(16%)	(24%)	(18%)	(15%)	(15%)	326,512 (19%)	(16%)
		Total	310,130	388,380	264,380	962,890	1,550,348	1,275,970		3,814,917
			(20%)	(19%)	(28%)	(22%)	(19%)	(17%)	988,599 (17%)	(18%)
Cows	2017	Farm	50,579	162,881	103,071	316,531	361,250	583,219		1,307,928
			(32%)	(18%)	(33%)	(25%)	(29%)	(15%)	363,459 (20%)	(21%)
		Market	81,998	9,820	33,104	124,992	327,193	31,189		572,382
			(16%)	(14%)	(24%)	(18%)	(15%)	(11%)	214,000 (22%)	(17%)
		Total	132,577	172,701	136,175	441,453	688,443	614,408		1,880,310
			(22%)	(18%)	(31%)	(23%)	(22%)	(15%)	577,459 (21%)	(20%)
	2018	Farm	49,680	169,137	93,736	312,553	311,543	589,387		1,220,692
			(30%)	(18%)	(29%)	(23%)	(30%)	(15%)	319,762 (18%)	(21%)
		Market	85,489	9,103	34,306	128,898	353,414	30,200		588,063
			(16%)	(12%)	(24%)	(18%)	(14%)	(10%)	204,449 (22%)	(15%)
		Total	135,169	178,240	128,042	441,451	664,957	619,587		1,808,755
			(21%)	(18%)	(28%)	(22%)	(22%)	(15%)	524,211 (21%)	(19%)

3.3. Annual network characteristics

3.3.1. Indicators and components

Out of the 1140 Ecuadorian parishes, 940 and 924 were implicated in cattle and cow trade in 2017 and 2018, respectively (Table 5). The percentage of links in the cow network corresponded to approximately 65% of the global networks links.

The global and cow networks showed a very low fragmentation, as the largest strong component included > 90% of nodes and the largest weak component included all nodes. (Table 5, Figure 7).

Table 5. Descriptive parameters and indicators for the global and cow networks in Ecuador in 2017 and 2018

	Global network		Cow network	
Year	**2017**	**2018**	**2017**	**2018**
Number of nodes	940	943	924	913
Number of links	17473	19261	11496	12526
Diameter	6	6	8	8
Density	0.02	0.022	0.013	0.015
Average degree	37.2	40.9	24.9	27.4
Reciprocity	0.39	0.39	0.38	0.39
Clustering coefficient	0.24	0.25	0.23	0.24
Average betweenness	0.0018	0.0018	0.0021	0.0021
Average path length	2.76	2.74	3.15	3.07
Assortativity	-0.15	-0.14	-0.17	-0.17
Strong components number	63	3941	90	6059
Largest strong component size (% nodes)	878 (93%)	905 (96%)	835 (90%)	855 (93%)
Average closeness for nodes of the largest strong component	0.37	0.37	0.33	0.33
Weak components number	1	1	1	1
Largest weak component size (% nodes)	940 (100%)	943 (100%)	924 (100%)	913 (100%)
Number of communities	302	253	16	15

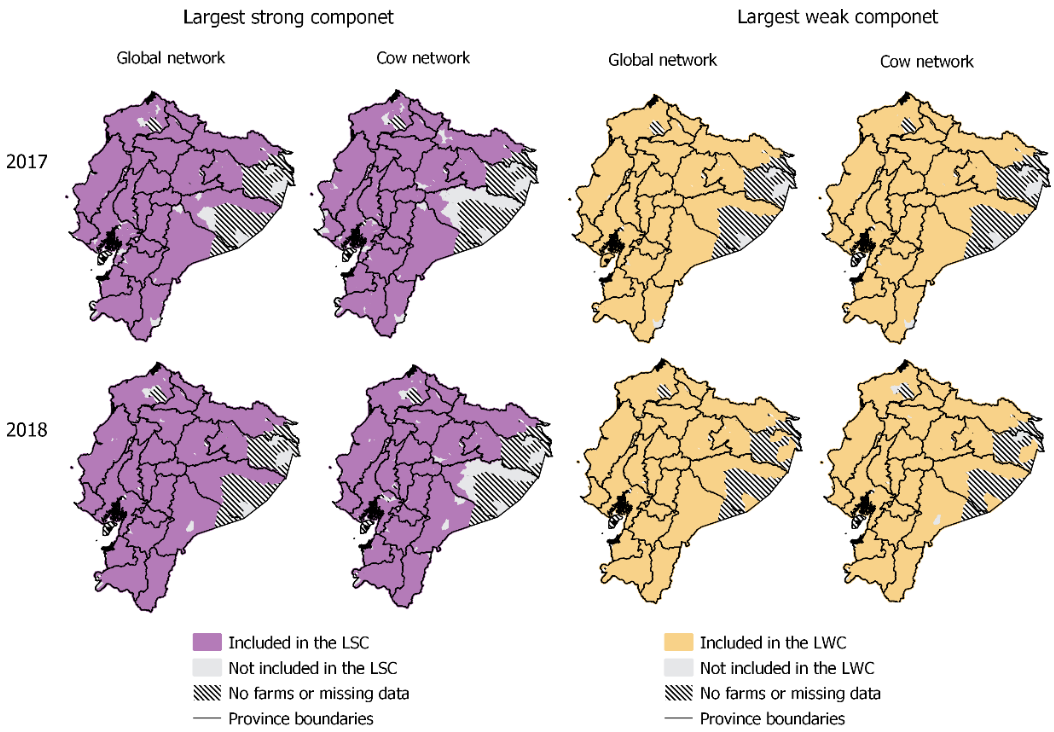

Figure 7. Spatial distribution of the largest strong and weak components for the global and cow networks in Ecuador in 2017 and 2018 (LSC: largest strong component, LWC: largest weak component)

As expected, density and average degrees were slightly higher for the global network compared to those for the cow network. Overall density values were low meaning cattle movements did not occur between many pairs of parishes. Reciprocity values showed that a little less than a half of parishes to which a given parish sends animals were the same from those from which it received animals. The degree distribution in a log-log scale did not show a linear shape for any of the networks (Figure 8).

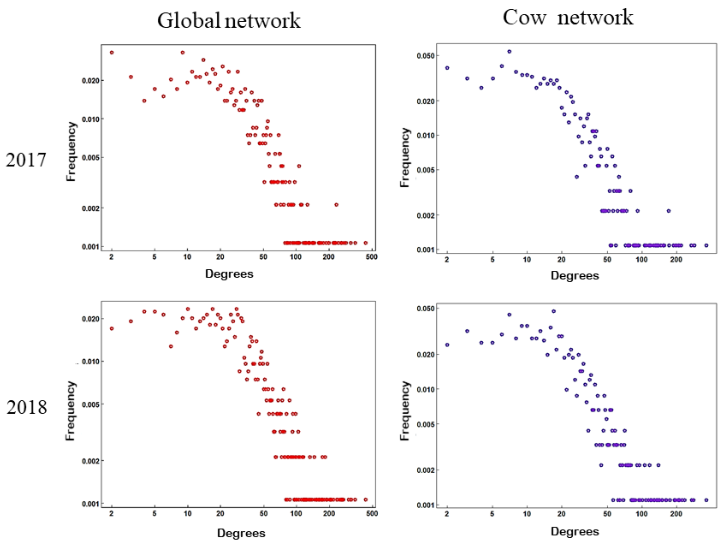

Figure 8. Degree distribution in a logarithmic scale for the global and cow networks in 2017 and 2018

Clustering coefficient and betweenness values were close for all four networks. The negative assortativity numbers indicated that the networks were disassortative. There was practically no change for the closeness among the annual networks (Table 5). Degree and betweenness were higher in parishes with animal markets than in parishes without markets (Figure 9).

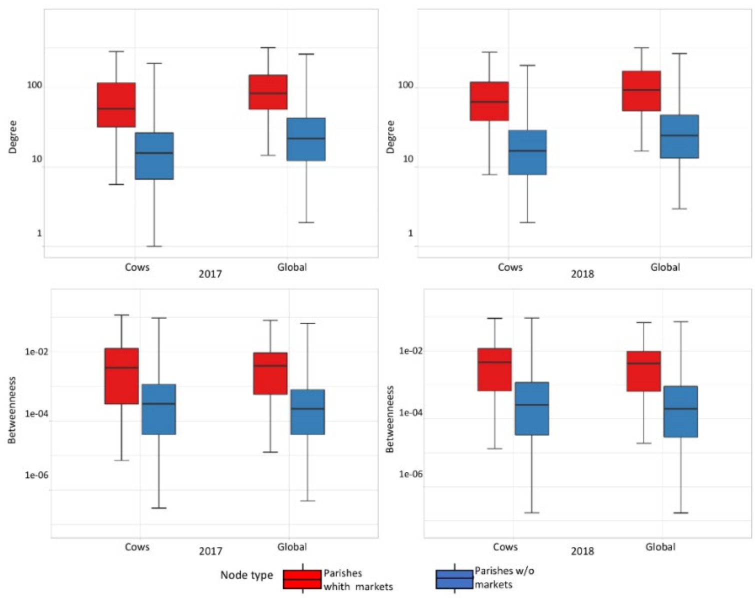

Figure 9. Distribution of degrees and betweenness in parishes with and without markets for the cow and global networks in 2017 and 2018

Clustering coefficient values were markedly higher (between 0.23 and 0.24, Table 4) than those of random networks having the same number of nodes and edges (0.04 in both years for the global networks, and 0.03 in 2017 and 0.028 in 2018 for the cow networks). Conversely, average path length values were close (between 2.76 and 3.15, Table 4) to those of the random networks (2.18 in 2017 and 2.12 in 2018 for the global networks, and 2.47 in 2017 and 2.39 in 2018 for the cow networks).

3.3.2. Communities

The global networks had a higher number of communities than the cow networks (Table 3). The first six largest communities grouped 93% (2017) and 97% (2018) of the parishes in the global networks and 84% (2017) and 91% (2018) in the cow networks. Spatial distribution of the first six communities (Figure 10) suggests that the spatial location and extent of the first three communities was close in the global and cow networks for both years.

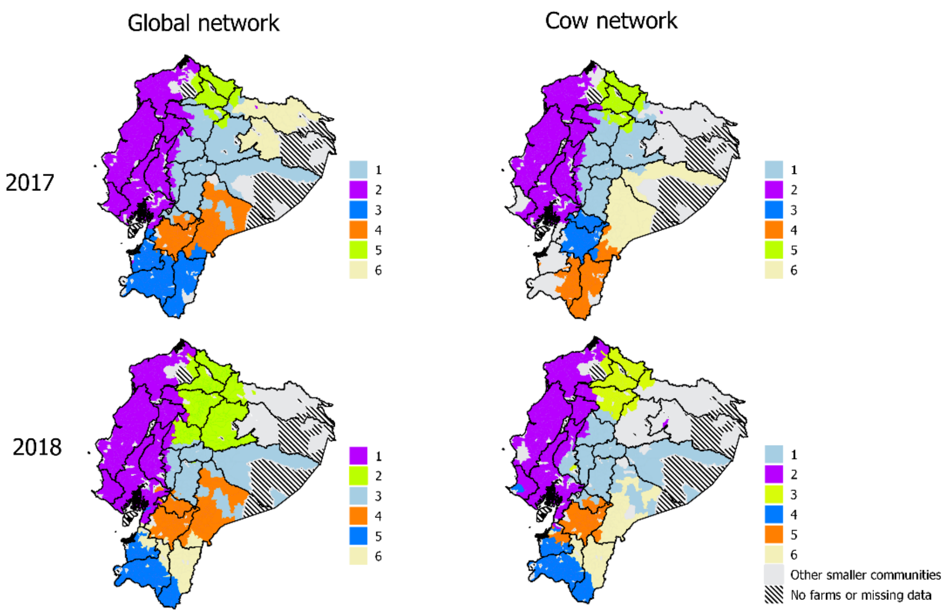

Figure 10. Spatial distribution of communities in the global and cow networks in 2017 and 2018

3.4 Monthly network indicators

Monthly indicators showed little variation from month to month for both years (Figure 11). Number of nodes, number of links, average degree and reciprocity were lower for cow networks than for global networks while average path length and average betweenness, for most of the months, were higher. Assortativity had negative values and, as for clustering coefficient, there were no remarkable differences for the two types of network.

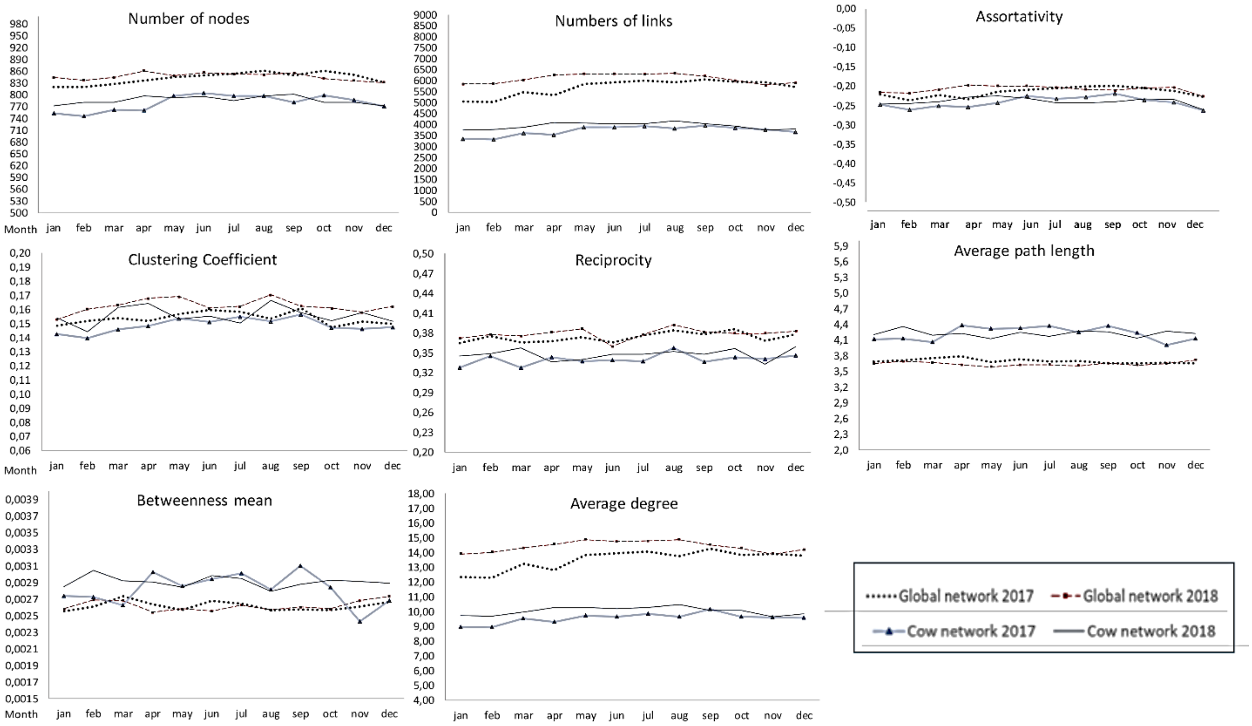

Figure 11. Monthly indicators of the global and cow networks for 2017 and 2018

3.5. Node and link trends in time

The Jaccard index for the annual global networks was higher for the nodes (97%) than for the links (40%). The annual cow network JI values were very close: 95% for the nodes and 39% for the links. In other words, practically the same parishes participated in animal trade in both years and only half of the exchanges occurred between the same parishes.

For the monthly networks the JI values fluctuated between 83% and 94% for the nodes and between 41% and 46% for the links (Figure 12).

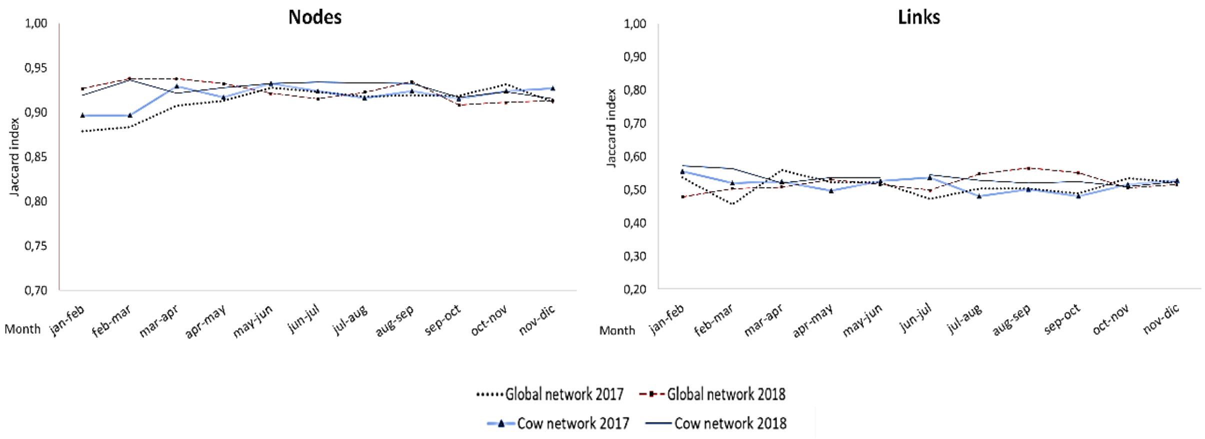

Figure 12. Jaccard index for the monthly global and cow networks in 2017 and 2018

3.6. Percolation analysis

The removal of parishes including markets did not break the largest strong component (LSC). For the two types of network, the LSC still represented between 80% in 2017 and 87% in 2018 of the total number of nodes.

Results of node removal based on their betweenness are shown in Table 6. For the four networks, a high number of nodes needed to be removed to attain the 10% threshold size of the LSC. Among the removed nodes with high betweenness there was a high percentage of parishes with markets.

Table 6. Percolation results based on the removal of nodes having the highest betweenness centrality, applying a 10% threshold size of the largest strong component for the global and cow networks in Ecuador in 2017 and 2018

	Global network		**Cow network**	
Year	2017	2018	2017	2018
No initial nodes	940	943	924	913
No total removed nodes* (% of initial nodes)	341 (37%)	357 (38%)	283 (31%)	287 (31%)
No removed nodes with higher betweenness having markets (% of nodes with markets)	45 (71%)	45 (71%)	42 (67%)	45 (71%)

*include removed nodes with higher betweenness and nodes that only had links with those nodes

4. Discussion

In this study, a description of Ecuador's cattle inventory was made using the data collected during the FMD vaccination campaigns. Cattle movement records for 2017 and 2018 were processed and studied using the SNA methodology and network indicators. Networks considering parishes as nodes and cattle movements as links were analyzed at the annual and monthly levels. We analyzed (i) the entire cattle movement database to document network characteristics relevant to the early detection and control of an epidemic disease such as FMD, and (ii) cow movement data, to describe network characteristics relevant to the surveillance and control of an endemic disease primarily affecting adult females, such as bovine brucellosis.

Ecuadorian cattle population figures estimated in our study from FMD vaccination campaigns were close for 2017 and 2018, and did not differ from those established in the 3rd national livestock census conducted in 2002 (Haro Oñate, 2003). They were also consistent with the results of a study that showed that the cattle population in Ecuador had not experienced changes in the last decade (Sánchez, A. M. et al., 2019). The decrease of cattle population size could be explained by the gradual decline in the red meat consumption, including the tendency to replace beef with other cheaper sources of protein (poultry and pigs).

The total number of mixed farms in Ecuador was at least three times higher than beef farms and five times higher than dairy farms, confirming that it is the most general category, as in other countries of South America (FEDEGAN, 2021, MINAGRI, 2017). Mixed production in Ecuador is related to small farms (1 to 20 ha) in which agriculture and dual-purpose livestock are developed with little technology, while specialized meat or milk production almost always corresponds to consolidated businesses (Haro Oñate, 2003). As a result, mixed systems mostly resort to self-sufficiency practices, unlike large farms (more than 100 ha) that use technological packages (nutritional, sanitary, and reproductive) and medium-sized farms (20 to 100 ha), which have technical assistance, although to a lesser extent.

We found that there was a higher density of farms and animals/km^2 in the Sierra region and that most of those farms were dairy farms. This finding is in accordance with the results of a study that pointed out that this region has many intensive farms specialized in dairy cattle raising (Requelme & Bonifaz, 2012) The highest density of mixed systems was found on the Coast region. In contrast, the density of farms and animals/km^2 in the Amazon region was very low very likely due to the fact that 53% of the land correspond to natural forests and woodlands (Ríos-Núñez & Benítez-Jiménez, 2015).

Markets appeared to play a major role in the organization of cattle trade, as half of the movements and moved cattle leaving farms were directed to markets rather than to other farms or to slaughterhouses. As there were not markets in all parishes, this finding is consistent with the fact that the vast majority of those cattle movements occurred between parishes.

Some movements from farm to farm could correspond to a form of seasonal transhumance that still exists in Ecuador among small producers consisting in the temporary use of pastures in the months of drought or shortage of pasture (Stewart et al., 1976). On average, seven cattle were transported per movement between farms. This figure is lower than the one reported by Aznar et al. (2011) in Argentina, where an average of 32 animals per movement between farms was reported in 2005, which is possibly explained by the largest size of the farms and higher magnitude of the trade.

One of our original purposes was to compare network indicators at the holding level and at the parish level. Unfortunately, networks could only reliably be built at the parish level.

Networks indicators were very close for global and cow networks at the annual and monthly scales. The degree distribution in a logarithmic scale did not show a linear trend for any of the annual networks, which is a property of scale-free networks. Conversely, the comparison with random networks of the same size (numbers of nodes and links) revealed properties of small-world networks: markedly higher values of the clustering coefficients in our networks than in random networks, whereas the average path lengths were similar. In small-world networks, low average path length values are indicative of a higher propagation speed of a disease (Shirley & Rushton, 2005). This could be the case in Ecuador if a FMD serotype/topotype not covered by the vaccines being currently used were to be introduced.

Furthermore, we found that more than 90% of the parishes were included in the largest strong (LSC) and weak (LWC) components. Transmissible cattle diseases such as FMD can spread rapidly over practically the whole network (Gábor Csárdi & Tamas Nepusz, 2005). The size of the largest components of trade networks could be considered helpful for analyzing the maximal extent of an epidemic: Kao et al. (2006) proposed that the size of the LSC is an estimate of the lower bound of the maximum epidemic size, while the size of the LWC is an estimate of its upper bound. However, the size of the LSC might not be the best measure to estimate the maximal size of an epidemic, since there should be a path formed by movements in the network leading back to the farm source of infection, and an epidemic does not follow this rule (Dubé C. et al., 2011). In the same way, the relationships between nodes are assumed to be reciprocal in the LWC (Dubé et al., 2009). Moreover, as highlighted by Rautureau et al (2010), when building the networks, movements between pairs of nodes are aggregated and their chronology is not taken into account. In any case, size and geographical extent of largest components represent a structural risk that should be considered when designing measures to mitigate an emerging disease spread.

All the built networks were disassortative (negative values) meaning that the parishes had more often links with parishes with a different degree. This finding can be explained by the fact that parishes with markets received animals from many parishes without markets. Degrees and betweenness, which are centrality network indicators, were higher in parishes with markets. This result suggests that in case of introduction of an infectious disease, the veterinary authorities should improve surveillance along the market path for early detection and reporting. Targeting nodes with high centrality values may allow implementing efficient measures of disease control and surveillance. However, neither of the two approaches we implemented to conduct percolation analyses (removal of nodes having higher degrees, parishes with markets, or higher betweenness) broke the LSC unless a large proportion of nodes were removed. This result may be due to the fact that the studied networks had no free-scale properties. Indeed, in previous studies, percolation analyses using centrality indicators to remove nodes were effective when holdings where the nodes and networks were scale-free. This was the case for livestock networks in France (removing 1% or 5% of premises achieved to break the LSC (Dutta et al., 2014; Rautureau et al., 2010), Italy (Bajardi et al., 2011), Germany (Büttner, 2013), and England (Kiss et al., 2006).

Finally, the low average path length values and high clustering coefficients values observed in our networks is a property of small-world networks, which implies that an endemic infection such as bovine brucellosis in Ecuador would spread within local clusters. We also found that the first six largest communities were spatially distributed in the same zones for all the annual networks indicating that movements within these regions were more frequent. This finding, as well as high reciprocity values and stability of nodes and links in time, suggest that bovine brucellosis may be spreading locally within those communities through cattle movements and that the transmission could be sustained in time. Unlike Colombia (a neighboring country), where vaccination against bovine brucellosis and a certificate for animal movement is mandatory throughout its territory (Avila-Granados et al., 2019), these measures have not yet been implemented in Ecuador. The identification of communities may allow the development of control strategies such as zoning and compartmentalization (WOAH, 2019) by subdividing into sectors the zones where movements are more frequent and could facilitate implementing a bovine brucellosis control program in Ecuador. Some experiences could be implemented in those communities according to their characteristics. For example, it has been possible to prevent and control zoonotic and high-priority diseases through the active participation of producers and key decision-making groups (Azhar et al., 2010).

Since livestock trade connects practically the whole country and a contagious disease could reach the whole territory, strategies such as compulsory vaccination and movement control of cattle should be implemented as it was the case for FMD. In the case of bovine brucellosis, it should also be considered to include the issuing of vaccination certificates as a requirement for trading cattle.

This work has allowed us to understand how two diseases, one of rapid diffusion and the other of slow diffusion, could spread through the movement of cattle at the parish level in Ecuador. However, we still have to understand if the results we obtained remain valid when the movements are analyzed on a smaller scale, that is, between farms.

5. Conclusion

Our analysis showed that cattle movement network in Ecuador using parishes as nodes had properties of small-world networks, both for all cattle and only cows. These properties may allow a fast spread of an epidemic disease such as FMD throughout the country, and also favor the local transmission of an endemic disease such as bovine brucellosis. They should be taken into account by the veterinary services when considering preventing measures in the case of an emerging disease or control measures for endemic diseases.

Chapter 2. Farm prevalence of bovine brucellosis, farmer awareness and local practices in small and medium scale cattle farms in a tropical region of Ecuador

1. Introduction

The province of Esmeraldas is one of the provinces on the Ecuadorian coast where bovine brucellosis prevalence is high. The scarcity of studies and the lack of updated data limits the understanding of the situation of bovine brucellosis in this province. Some investigations on risk factors had been conducted in nearby provinces in medium to large production systems. (Carbonero et al., 2018; Zambrano Aguayo et al., 2016) but not in Esmeraldas province.

In general, in Ecuador, very few published studies have addressed the problem of bovine brucellosis in small farms that represent more than 70% of the total number of farms in the country (INEC-ESPAC, 2019). Besides, little is known about the habits and customs of the population in this sector regarding herd management that could be a risk for public health.

Quinindé is one of the cantons of Esmeraldas province where cattle raising for meat and milk production constitutes the most important economic activity. No information was available on the bovine brucellosis prevalence in this canton and no studies on risk factors had been implemented.

We conducted a study in dairy and mixed small- and medium-scale farms in Quinindé canton that aimed: i) to estimate the prevalence of bovine brucellosis at the farm level in small and medium scale cattle farms; ii) to identify potential risk factors associated with bovine brucellosis positive farms or farms with reproductive disorders, iii) to evaluate the knowledge of the disease among farmers, and iv) to analyze the risk that bovine brucellosis on these farms could represent for public health. The results of this study are described in this chapter.

2. Material and methods

2.1. Study area

Quinindé is one of the seven cantons (intermediate administrative unit) in the Esmeraldas province (Ecuador is divided into 24 provinces) located in the coastal region at 190 meters above the sea level. Its temperature oscillates around 25°C, with a relative humidity above 80%. Around half of the farms and cattle population of Esmeraldas province are located in Quinindé (~ 4,200 farms and 135,000 cattle). Most of the farms are mixed farms (cultivation of crops combined with cattle farming) of dual purpose raising zebu (*Bos indicus*) and its crosses with European breeds.

2.2. Study design

The study was conducted between March and November 2017.

For the sampling size estimation, a between-farm prevalence of 11%, a confidence level of 95% and a precision of 5% were considered. A sample size of at least 135 cattle farms was obtained using the WIN EPI 2.0 program (De Blas et al., 2006). We looked for increasing that number to at least 170 farms to conduct the identification of risk factors. The farm census information from the Foot and mouth disease Information System of Ecuador was used for the farm selection. Of 4,200 small cattle farms present in Quinindé, 1,772 were retained for the random sampling because they were small or medium farms and had a record of dairy or dual-purpose activity.

2.3. Milk sampling and laboratory analysis

Milk samples of 100 ml were taken in each farm from the milk collection tanks, and kept in sterile jars. The samples were collected at milking in the morning in their raw state, making sure not to contaminate the contents. The samples were transported in refrigerated thermal boxes, maintaining the cold chain, to the laboratories of the Universidad San Francisco de Quito, where they were stored at -20 ^{O}C, until the date of shipment to the National and WOAH Animal Brucellosis Reference Laboratory, Anses, located in Maisons-Alfort, France, where they were analyzed.

An antibody test was performed using and indirect ELISA Brucellosis Milk Ab Test from IDEXX. The short incubation protocol was performed following the procedures described by the manufacturer.

2.4. Survey design

A questionnaire including multiple choice and open questions was designed to collect information on the following topics: farm characteristics, veterinary assistance, herd management, milk and dairy products trade, milk and cheese consumption, animal health management and farmer brucellosis awareness.

The questionnaire was first validated within a group of 10 farmers from a neighboring canton. Following this validation, some questions were adjusted to facilitate the understanding of the farmers of the area.

The information was collected with the support of final year veterinary students and local health technicians, who received prior training.

2.5. Statistical analysis

Between-farm apparent prevalence was calculated based on the ELISA results.

Two multivariable analyses were conducted using a logistic regression to identify risk factors linked to milk seropositivity on the one hand, and, on the other hand, farmer reporting of reproductive disorders. The bovine brucellosis serological status of the farm (i.e. the ELISA result obtained from the milk collected in the farm) and the occurrence of reproductive disorders were the binary outcomes. The farm size was categorized into two categories: small farms (< 50 cattle) and medium farms (≥ 50 cattle).

Sixteen variables were considered for the identification of risk factor linked to farm status (Table 7). Multicollinearity between these variables was checked to ensure a variance inflation factor (VIF) < 2. The procedure to select the variables for the final multivariable model is described in (Hosmer D., 2000). Briefly, univariable associations were tested and the variables with $p < 0.2$ were retained to be included in the multivariable analysis. The least significant variables were then removed using a backward and forward stepwise procedure. Only variables with $p < 0.05$ were retained in the final model.

Concerning the occurrence of reproductive disorders, bovine brucellosis status of the farm and farm size were considered as risk factors and tested directly in a multivariable analysis.

Statistical analysis were performed using the RStudio software version 1.4.1717 and the epiR, car, and MASS packages (R Core Team 2021). The georeferenced maps were obtained in shape file format and scale 1:50,000 from the Geoportal of the Ecuadorian Military Geographic Institute (http://www.geoportaligm.gob.ec/), and processed in Qgis 3.6.0 Noosa (QGIS.org, 2020).

3. Results

3.1. Sample description

The farm sample was composed of 131 small farms (1 to 50 cattle) and 42 farms of medium size (51 to 200 cattle). One-third of the surveyed farms reported cattle raising as the main economic activity (35/131 small farms, 34/42 medium farms, Fisher's exact test: $p < 0.0001$); all the others had also agriculture activities. The average surface was 22 hectares for small farms, and 33 hectares for medium farms.

All farms managed cattle extensively, with cultivated pastures. In most cases, pastures were subdivided for rotational grazing. In times of drought, some farmers rented pastures (37/131 small, and 12/42 medium; Fisher's exact test: no significant difference [NS]) and some of those with irrigation rented their pastures and received animals from other farms.

All herds were of mixed type. The proportion of dairy cows was higher in the medium farms than in the small ones (22% in medium farms, 6% in small farms; Fisher's exact test: $p < 0001$). Almost all farms had other animal species: horses/mules (147/173), pigs (72/173) and dogs (160/173). Sheep or goats were not reported on any of the farms.

None of the farms visited had potable water. A minority of farms had non-potable piped water (40/131 small farms, 22/42 medium farms; Fisher's exact test: $p = 0.03$), while the others had access to sources such as wells and reservoirs or streams shared with neighbors.

Most farmers replaced their animals with livestock from their own farm (101/131 small farms, 25/42 medium farms; Fisher's exact test: $p = 0.03$), and the others bought animals at markets or from neighbors. Seventy percent of farmers sold their cattle to traders that came to the farm and 29% at markets or to neighbors.

Natural breeding was implemented in 169 farms (127/131 small farms, 42/42 medium farms; Fisher's exact test: NS), most of the times using their owned breeding bull. Artificial insemination was implemented in 3 small and 4 medium farms. Calving occurred in dedicated pens in 17/173 farms (5/131 smalls farms and 10/42 medium farms; Fisher's exact test: $p < 0.0001$).

Only a small group of farmers (8/131 small-scale farmers, 9/42 medium-scale farmers; Fisher's exact test: $p = 0.007$), belonged to a cattle association. One-third of the farms (33/131 small farms, 26/42 medium farms; Fisher's exact test: $p < 0.0001$) had veterinary assistance. This technical veterinary assistance was provided mainly by state organizations (mainly for the small farms: 25/33) or veterinary companies (mainly for the medium farms: 11/26).

3.2. Farm apparent prevalence estimation

The overall apparent between-farm prevalence was 11.5% (95% IC: 6.7%-16.2%). In the group of medium farms, the between-farm prevalence (23.8%, 95% IC: 10.9% - 36.6%) was significantly higher (Fisher's exact test: $p = 0.02$) than in the group of small farms (7.6%, 95% IC: 4.5%-9%). The spatial distribution of farms according to their ELISA result is shown in Figure 13.

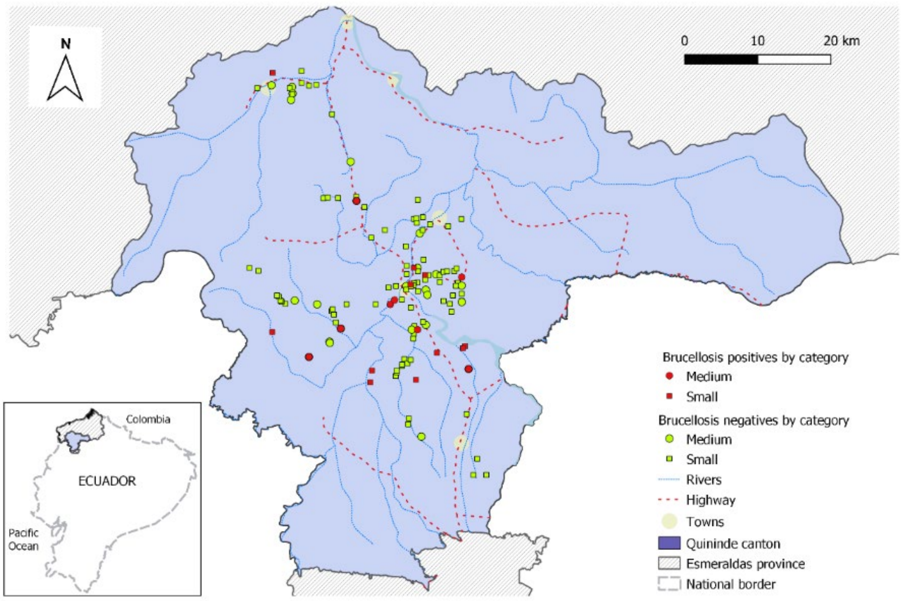

Figure 13. Brucellosis-positive and -negative cattle farms according to farm brucellosis serological result in milk tank from 131 cattle farms sampled in Quinindé canton, province of Esmeraldas, Ecuador

3.3. Risk factor analysis for bovine brucellosis status of farms

The 16 potential risk factors for bovine brucellosis included in the univariable analysis are described in Table 7 according to the farm brucellosis status. No collinearity was detected among those variables.

Table 7. Descriptive statistics of potential risk factors included in the univariable analysis according to farm brucellosis serological result in milk tank from 131 cattle farms sampled in Quinindé canton, province of Esmeraldas, Ecuador

Features	**Potential risk factor**	**Category**	**Positive/total (%)**
Cattle replacement	Farm size*	Small	10/131 (8%)
		Medium	10/42 (24%)
	From neighboring farms	yes	3/29 (10%)
		no	17/144 (12%)
	From the same farm*	yes	12/126 (10%)
		no	8/47 (17%)
	Purchased at fairs*	yes	6/24 (25%)
		no	14/149 (9%)
Calving	Having calving pens	yes	1/17 (6%)
		no	19/156 (12%)
Reproduction	Natural breeding	yes	19/169 (11%)
		No	1/2 (50%)
	Insemination	Yes	0/7 (0%)
		No	20/166 (12%)
Abortion management	Incineration/burial of abortion material*	Yes	9/115 (8%)
		No	11/58 (19%)
	Keeps the aborting cows	Yes	17/140 (12%)
		No	3/33 (9%)
Manure management	Leave manure in the barn	Yes	13/119 (11%)
		No	7/54 (13%)
	Use manure as fertiliser	Yes	4/44 (9%)
		No	16/129 (12%)
	Manure runs off into a stream	Yes	2/10 (20%)
		No	18/163 (11%)
Share of pastures and water sources	Pastures shared with cattle from other farms	Yes	15/146 (10%)
		No	5/27 (19%)
	Water source shared with other farms*	Yes	12/75 (16%)
		No	8/98 (8%)
Others	Share livestock utensils with other farms	Yes	1/3 (33%)
		No	19/170 (11%)
	Cattle vaccination against brucelosis (RB51 Strain)	Yes	0/1 (0%)
		No	20/172 (12%)

*Variables found significant at $p < 0.2$ in the univariable analysis

In the multi-variable analysis, farm size and incineration/burial of abortion material were identified as significantly associated with farm brucellosis status (Table 8). Medium farms had 3.7 more odds to be infected than the small farms (OR: 3.7, 95% CI: 1.39 - 9.84, $p = 0.008$). The incineration or burial of the abortion material was a protective factor (OR: 0.4, 95% CI: 0.14 - 0.98, $p = 0.04$). When not incinerated or buried the abortion material was in general left in the pastures or could also be given to the farm dogs.

Table 8. Results of the multivariable logistic regression on the farm brucellosis status according to serological result in milk tank from 131 cattle farms sampled in Quinindé canton, province of Esmeraldas, Ecuador

Risk Factors	*Categories*	*Odds ratio*	*95 % CI*	*p-value*
Farm size	Small	Reference		
	Medium	3.7	1.39 - 9.84	0.008
Incineration/burial of abortion material	Yes	0.4	0.14 - 0.98	0.04
	No	Reference		

3.4. Risk factor analysis for the occurrence of reproductive disorders in farms

Reproductive disorders were reported in 23 medium farms (55%) and 32 small farms (24%). Farm size and brucellosis-positive status were found to be associated with their occurrence (Table 9). The odds of reproductive disorders was 2.9 times higher in medium-sized farms than in small farms (OR: 2.9, 95% CI: 1.07 - 8.06, $p = 0.002$). Reproductive disorders were reported in 12 farms positive for *Brucella* (60%) and 8 farms negative for *Brucella* (40%). The farms positive for brucellosis had 3.3 higher odds of having reproductive disorders than the negative farms (OR: 3.3, 95% CI: 1.55 - 6.89, $p = 0.008$).

Table 9. Results of the multivariable logistic regression model on the occurrence of reproductive disorders in 131 cattle farms sampled in Quinindé canton, province of Esmeraldas, Ecuador

Risk Factors	**Categories**	**Odds ratio**	**95 % CI**	***p*-value**
Farm size	Medium	2.9	1.07 – 8.06	0.002
	Small	Reference		
Brucellosis positive farms	Pos	3.3	1.55 - 6.89	0.04
	Neg	Reference		

3.5. Awareness of farmers about brucellosis

Forty-four of the 173 farmers surveyed (i.e. 25%) mentioned having heard about the disease (29/131 small-scale farmers and 15/42 medium-scale farmers, Fisher's exact test: NS). However, only 15 of these 44 farmers were aware of the brucellosis transmission route between animals, the proportion being significantly lower in small-scale farmers (5/29) than in medium-scale farmers (10/15) (Fisher's exact test: $p = 0.002$). The proportion of farmers who knew that the disease was zoonotic among those 44 farmers was significantly higher in medium-scale farmers (10/15) than in small-scale farmers (7/29) (Fisher's exact test: $p = 0.009$).

Vaccination against brucellosis was performed in four farms (1/131 small-scale farms, 3/42 medium-scale farms) (Fisher's exact test: p= 0.044). The strain 19 vaccine was applied in one small-scale farm and in three medium-scale farms. Strain RB51 vaccine was also used in one medium-scale farm.

Ninety-eight of 173 farmers (57%) showed interest in participating in a control and prevention plan and 21/173 (12%) considered the process too complicated. Seven farmers were not interested in participating in a control and prevention plan because they assumed it would be too expensive.

3.6. Public health risk

Fresh milk was marketed directly or through intermediaries on 36/131 small farms and 19/42 medium farms (Fisher's exact test: $p = 0.04$). Of these farms, seven were positive for bovine brucellosis. Raw milk was occasionally consumed in 10 small farms (2 were positive for bovine brucellosis) and seven medium farms (2 were positive for bovine brucellosis) (Fisher's exact test: NS). Curdled unpasteurized milk was regularly consumed on 112 farms (87/131 small farms, 25/42 medium farms) of which 14 were positive for brucellosis (4/87 small farms, 10/25 medium farms; Fisher's exact test: $p < 0.0001$).

Cheese was produced on 153/173 farms (117/131 small farms, 36/42 medium), of which 18 were bovine brucellosis positive (8/117 small farms, 10/36 medium farms; Fisher's exact test: $p = 0.001$). It was sent to markets by 83 farms (57/131 small farms, 26/42 medium farms), of which 17 were positive farms (8/57 small farms, 9/26 medium farms; Fisher's exact test NS), or consumed on 156 farms (119 small farms, 37 medium farms), of which 18 were positive (8/119 small, 10/37 medium; Fisher's exact test: $p = 0.002$). The cheese was produced using traditional methods, and none of the farmers reported cheese pasteurization.

Four small farmers reported that they consumed the aborted fetuses.

Six medium-scale farmers reported having had a family member diagnosed with brucellosis in the last five years (none among the small-scale farmers, Fisher's exact test: $p = 0.002$).

4. Discussion

Bovine brucellosis in Ecuador has not been sufficiently studied. As in other Latin American countries, bovine brucellosis continues to be underdiagnosed and underreported (Godfroid et al., 2011; López-Merino, 1989), so its prevalence is probably underestimated. On the other hand, most studies on bovine brucellosis in Ecuador have been carried out in industrial and semi-industrial milk production systems, which constitute barely 30% of the country's total livestock farms. No studies on bovine brucellosis had been conducted previously in small and medium-sized farms that represent 92% of the total productive units registered in the country (Haro Oñate, 2003). These farms are considered family farms of dual-purpose (agriculture and livestock raising) with little technology (Craviotti & Schneider, 2014). The aims of our study were to provide information on brucellosis prevalence, risk factors, level of knowledge of the disease among farmers and identify risk practices in small and medium farms in a tropical region of Ecuador.

We found an overall farm bovine brucellosis prevalence of 11.5%. This value is below the one reported (45%) in a study carried out in commercial dairy and mixed farms in the Ecuadorian central provinces (Carbonero et al., 2018) or in a study conducted in commercial mixed cattle farms (22%) in Manabí province (Zambrano Aguayo & Pérez Ruano, 2015). In neighboring countries, such as Colombia, where surveys have been conducted in productive farms of the main dairy regions, prevalence values between 22% and 43% were reported at the herd level depending on the region (Cárdenas, 2018; Motta-Delgado et al., 2018).

We also found that the bovine brucellosis prevalence in medium-sized farms (23.8%) was significantly higher than in small farms (7.6%). In addition, the size of the farm was identified as a risk factor for the presence of brucellosis. It has also been the case in other studies leading the authors to point out that the largest farms are more exposed to the disease due to more cattle movements from or to markets or to the introduction of new animals for growth or fattening (Al-Majali et al.,2009; Chand & Chhabra, 2013; Matope et al., 2011). In Ecuador, even though in the Manual of procedures for the control of bovine brucellosis it is established that moved cattle needed a health certificate showing that they are free from brucellosis (Agrocalidad, 2016), in practice, most farmers do not require this document for transactions. Therefore, the probability of introduction of the disease into a bovine brucellosis free farm is higher for farms purchasing more animals (Vinueza et al., 2022). The medium size farms included in our study did not have the technology level of big commercial farms, but they were more often involved in livestock trading than the small farms. A similar situation occurred in the largest herds of dairy goats in Ecuador where higher prevalence values of bovine brucellosis were found (Poulsen et al. 2014).

Burying/incinerating fetal remains was identified as a protective factor in our study. The survival of *Brucella* in the environment has been reported in several studies (Castro et al., 2005; El-Sayed & Awad, 2018). Specific characteristics allow the pathogen to survive in moist soil at room temperature for up to 66 days, in manure for up to 80 days, in leather stained with cow dung for 21 days, and even in water at 8 °C and a pH of 6.5 for more than 57 days (Castro et al., 2005). Some authors claim that *Brucella spp*., can resist in the soil with a relative humidity of 40% for several months at 4-8°C, and 144 days at 20°C (Bercovich, 1998). Other authors have pointed out that an average temperature of 18 °C and a high relative humidity may favor the persistence and spread of the disease (Dadar et al., 2020). Those humidity and temperature conditions are fully met in the tropical zone of Ecuador. In the current study, it was observed that it is a common practice for some farmers to leave the remains of abortions in the pasture or to allow their access to dogs. This practice has also been reported in other studies as a risk factor (Deresa et al., 2020). Moreover, dogs have been shown to act as reservoirs and vehicles for the spread of *Brucella abortus* on livestock farms (Wareth et al., 2017), reason why some authors suggest that dogs could also be sentinels for the detection of this disease (Baek et al., 2003). Well-managed breeding systems (including isolation of aborting animals) can help reduce the prevalence of infection within the herd (Salman et al., 1984) and proper disposal of aborted materials and highly hygienic procedures constitute crucial steps in any successful *Brucella* control program (Al-Majali et al., 2009).

Some risk factors included in our study were not significant but should be considered for the proper control of the disease, among them: calving in the open field, the fate of the cow that aborts, the management of manure, the origin of the animals, the use of ditch water from other farms, the lack of vaccination and immunity of livestock, the type of reproduction and the biosecurity measures applied at the farms, and others (Avila-Granados et al., 2019).

Brucellosis and farm size were linked to the occurrence of abortions in our study. In areas where brucellosis is endemic, abortions and retained placentas may raise suspicion about the presence of the disease in the first place, mainly when abortion occurs in the last third of pregnancy (Calistri et al., 2013; Guzmán-Hernández et al., 2016). However, it should be taken into account that not all abortions can be attributed to brucellosis, and not all animals infected with brucellosis will necessarily abort. Abortions in Ecuador could also be attributed to other infectious agents such as infectious bovine rhinotracheitis, bovine viral diarrhea or leptospirosis (Vargas-Niño et al., 2018; Macías et al., 2019).

Different strategies have been undertaken in Ecuador to control brucellosis. Some have been successfully implemented in commercial farm but they have not reached the small producer sector. It could be due to the lack of official technical assistance. It has been reported that less than half of the medium farms in Ecuador have access to technical assistance (Torres et al., 2015). In this study, it was found that only a third of farms had access to technical assistance and that in most cases it was provided by veterinary commercial companies whose interests may not include the official sanitary measures guidelines.

In our study, only 11% of producers knew how bovine brucellosis was transmitted to cattle. Although some producers had heard of the disease, most did not understand its transmission mechanism or that it was a zoonosis. This brucellosis unawareness would not be limited to this region. Indeed, in a study carried out in the Ecuadorian Andean region, it was pointed out that indigenous communities were unaware of the disease (Uvidia et al., 2018). In another study including 500 farmers of the province of Manabí in Ecuador, only 30% knew about brucellosis, and 7.6% about the measures to reduce its transmission (Pérez Ruano & Aguayo, 2017). The lack of knowledge of the disease among people involved in bovine production (farmers, slaughterhouse workers, cattle and milk dealers) has also been reported in other regions of the world. In a meta-analysis including 22 countries in Asia, Oceania, Africa, North, South and Central America it was found that only 35% of the interviewees knew about the disease, and 36% that it was a zoonosis (Zhang et al., 2014). Brucellosis awareness could also be improved through the participation to farmers' associations. In our study, although there was a significant difference between the two groups of farmers, few of them belonged to a cattle association. On the other hand, half of the farmers showed interest in participating in a prevention program but some found the process complicated or too expensive. Therefore, different aspects may be playing a role in the lack of interest of small- and medium-scale farmers from the study region to participate in the national brucellosis control program that include low brucellosis awareness, absence of economic incentives and lack of guidance/support from the authorities.

In the Ecuadorian coastal region, the consumption of raw curdled milk and fresh cheese made on the farm is a typical practice. The commercialization of fresh milk through intermediaries was significantly higher in the group of medium-scale farmers than in the small ones. The lower participation of small-scale farmers in milk trade could be explained by their low production yields and little bargaining power with intermediaries (Nieto Albuja et al., 2002). The more intense exchange by medium-scale farmers also means that milk from farms where brucellosis prevalence is higher could be reaching places farther from production centers. The significant lower participation of small-scale farmers in cattle associations could be affecting their access to the market since the associations can obtain better prices for their products.

Considering that a third of the farmers interviewed in this study marketed curdled milk and about 90% fresh cheese, including those whose farms were positive for brucellosis, this practice constitutes a danger not only for producers but also for those who consume the products in urban centers. In Colombia, the presence of *Brucella* has been confirmed in samples of fresh artisanal cheese (Soto-Varela et al., 2018). In an outbreak of *Brucella melitensis* in humans reported in Spain (Castell J, 1996), 81 human cases had a strong association with the consumption of fresh cheese made by an artisan in an establishment without control. In Ecuador, the presence of *Brucella* in products such as cheese has not been studied. Some of the interviewed farmers also reported the consumption of aborted fetuses. The consumption of bovine placenta or aborted fetuses is an alimentary tradition in some regions of Ecuador and has been linked with the occurrence of human cases of brucellosis not through the consumption of the food item, which is cooked, but due to its manipulation (Ron-Román et al., 2014). Although human brucellosis is considered primarily an occupational zoonosis among farmers, veterinarians and slaughterhouse workers, the consumption and handling of contaminated food or remains of abortions, and poor hygiene, can significantly increase the risk of the disease in the general population (Hegazy et al., 2011). In our study, six medium-scale farmers mentioned having had a relative affected by brucellosis in the last five years. Considering that bovine brucellosis prevalence was significantly higher in this category of farms, people living in these farms would be more exposed to brucellosis infection. Finally, human brucellosis underdiagnoses cannot be excluded in the region. Some authors have pointed out that in many cases, the symptoms of brucellosis in humans can be mild or infrequent, so the diagnosis is not even considered (Corbel, 1997). Additionally, in tropical countries such as Ecuador, some symptoms of brucellosis in humans can be confused with malaria and typhoid fever (Zhang et al., 2014).

5. Conclusion

Bovine brucellosis circulates in small- and medium-sized farms in a tropical zone of Ecuador with a higher prevalence in the medium-sized farms. The lack of bovine brucellosis awareness as well as some local practices that may influence the spread of the disease in cattle and humans were identified. For these reasons, to control the disease, it is necessary to make small and medium-scale farmers aware of brucellosis through information and health education campaigns. Warnings about the consumption of raw milk/cheese or the manipulation of aborted fetuses should be included in these campaigns. This first approach in the control of the disease is necessary to make acceptable other control measures, including vaccination.

Chapter 3. Awareness and risk perception of small- and medium-scale farmers and criteria of veterinarians about bovine brucellosis in Ecuador

1. Introduction

Despite the national bovine brucellosis control program implemented in 2008, the disease is still enzootic in Ecuador (Agrocalidad, 2019; Carbonero et al., 2018). Epidemiological studies on bovine brucellosis conducted in the country have mainly focused on estimating the disease prevalence, and in some cases identifying risk factors (Carbonero et al., 2018; Poulsen et al., 2014). Studies carried out in some areas where bovine brucellosis is present report a lack of knowledge about the disease among the population of up to 100% (Zambrano Aguayo et al., 2016; (Uvidia et al., 2018; Pérez Ruano & Aguayo, 2017). However, little is known about the level of awareness and risk perception of the farmers, nor have been documented the opinions of the veterinarians about the disease control strategies. Some authors consider that the effectiveness of the measures applied to control bovine brucellosis depends significantly on increasing farmer awareness to achieve safe herd management practices (Mantur & Amarnath, 2008). Awareness among farmers can be achieved through the implementation of education programs that require the participation of veterinary practitioners as well as veterinary officers.

Qualitative methods are mainly used in social disciplines to understand how individuals think, feel or behave in particular situations, or in relations with others. They include interviews, focus groups, participant observation, ethnography, historical analysis, and textual or content analysis. Focus groups allow the collection of information from a direct source and the investigation of in-depth knowledge. This depth is achieved thanks to the free expression and self-explanation of the participants about their experiences, perceptions and beliefs (C. Ivankovich-Guillén & Araya-Quesada, 2011). One of the advantages of focus groups is that they facilitate the participation of people who cannot read or write or who are reluctant to be surveyed or interviewed on their own. Focus groups have been implemented in epidemiological research in Ecuador regarding perception of risk and behavior in relation to zoonoses (Villacé et al., 2018) or antibiotic use in animal production (Waters et al., 2022). Some studies have been conducted to understand human population behavior in relation with bovine brucellosis (Adesokan et al., 2013; Díez & Coelho, 2013; Mendoza de Arbo et al., 2018).

The objectives of the study described in this chapter were to establish, through the focus group technique, i) the perception of issues on bovine brucellosis control in Ecuador in veterinarians involved in animal health in cattle farms and, ii) the awareness and risk perception of bovine brucellosis in small and medium-scale farmers.

2. Material and methods

2.1. Study design

The study was carried out based on the principles of the grounded theory proposed by Glaser & Strauss (Glaser & Strauss, 2010) and the methodology proposed by PAHO/WHO (Ulin et al., 2006) for qualitative research through focus groups, which includes purposive sampling, compilation, coding, and identification of thought patterns on the criteria exposed by a focus group.

Four focus group meetings of veterinarians and farmers were held from March to May 2022. The free, voluntary, anonymous and confidential nature of participation was explained at the beginning of the meetings so that attendees could express themselves freely. The study methodology was then introduced and consent was requested to record the session, guaranteeing the confidentiality of the source. The participants were informed that the results of the meetings would be published in an academic publication, preserving the anonymity of the attendees at all times. Finally, a commitment was made to share the investigation results, especially the conclusions and recommendations. In the farmer focus groups, details about brucellosis were given at the end of each meeting.

The meetings were guided by a single moderator. The participation of all members was encouraged with directed questions. In some cases, the moderator intervened in the discussion to avoid deviations and return to the topic. The discussion was allowed to continue until reaching saturation.

2.2. Focus groups of veterinarians

As we intended to collect information from veterinarians working in different fields, two focus groups were gathered: one from the official veterinary services including 3 local veterinary officers and 2 veterinary officers from the general directorate and one group including 5 veterinary practitioners and 4 working at universities. They were contacted by telephone and e-mail. Virtual meetings were held, which allowed the participation of veterinarians working in 8 of the 20 provinces of the country.

A bank of questions on bovine brucellosis issues was previously established considering the different fields of work of the veterinarians. The questions aimed to identify the perception of the brucellosis situation on cattle farms and the effectiveness of the brucellosis control plan in Ecuador.

The meetings started with two trigger questions:

- *What situations interfere with the control of bovine brucellosis in cattle farms in Ecuador?*
- *What actions should be carried out to adjust the strategy of the bovine brucellosis control institution in Ecuador?*

Several cross-questions were formulated to maintain the topic of discussion until saturation was reached.

2.3. Focus groups of farmers

Two groups of farmers from two different socio-productive strata were selected in two geographical regions (Figure 15). The first group included 12 indigenous farmers from Cotopaxi province in the Sierra region, who owned small family cattle farms. In the second group, we gathered 8 medium-scale cattle farmers from Manabí province in the Coast region. The farmers were contacted with the help of indigenous community leaders and leaders of farmer associations by telephone. Face-to-face meetings were held locally. In the case of the indigenous group, the indigenous leader, who was not a farmer, attended the meeting and, when necessary, made translations into Kichwa language.

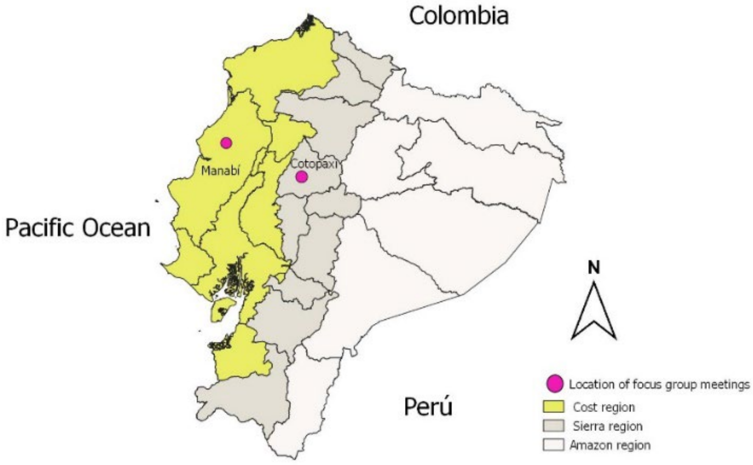

Figure 14. Locations where the meetings of the focus groups of farmers were conducted.

The purpose of the research and the interest in gathering criteria on the bovine brucellosis problems in the Ecuadorian rural sector were disclosed at the beginning of the meetings. Questions were addressed to estimate the level of knowledge on bovine brucellosis, awareness and risk perception.

Triggering questions were oriented to address three *a priori* categories:

i. The level of knowledge about the routes of transmission bovine brucellosis, and the adverse effects of the infection in animals and humans. The triggering questions were:

- *Are there diseases that can cause abortion in cows?*
- *Are there cattle diseases that can be transmitted to humans?*

ii. The level of awareness and risk perception of bovine brucellosis:

- *When a cow aborts, what should be done with the aborted fetus and the cow?*
- *Are the cows that abort kept on the farm?*
- *What do you do with the aborted fetus?*
- *Do you consume raw milk?*
- *Could an abortive disease such as bovine brucellosis ever affect your cows?*

. The application of measures focused on the prevention of bovine brucellosis:

- *When you buy cattle, do you ask for health certificates or try to find out if they have had diseases?*
- *When an animal is introduced into the farm, do you separate it for a while?*
- *Do you vaccinate your animals?*

At the end of the meetings, three veterinary students applied a small survey to the participants to obtain basic information on the productive characteristics of the farms.

2.4. Data processing

A data reduction process was conducted through encoding methods until the generation of categories according to the following steps:

a) Transcription of recordings to text and classification in the *a priori* categories
b) Thematic reading and open coding
c) Relational reading and axial coding
d) Selective coding

a) Transcription of recordings to text and identification of the a priori categories

In this first stage, the research material was processed and the parameters for the analysis of the data collected were established. The information collected through audio recordings was converted into text. From the *a priori* categories that were established based on the objectives of the study, descriptors were elaborated that facilitated the identification of text segments (Table 10).

Table 10. A priori categories and descriptors

Focus group	*Objectives*	*A priori categories*	*Descriptors*
Veterinarians	To establish the elements that hinder the implementation of an effective control program	Experiences on brucellosis with cattle farmers Experiences on implementation of the brucellosis control program in the field	Daily experience Personal or collective experiences Memories of relevant events
Farmers	To establish the level of knowledge regarding brucellosis To assess the level of awareness and the risk management of the likely occurrence of brucellosis on the farm	Experiences about abortions in cattle and the occurrence of bovine brucellosis Attitude towards the possible occurrence of the disease	Individual or collective experiences related to bovine brucellosis or abortion Importance given to the prevention or control of bovine brucellosis. Memories of relevant events related to bovine brucellosis Everyday cattle handling that could be dangerous

b) Thematic reading and open coding

In this second stage, the transcribed information was analyzed by approaching each a priori category individually. Each idea or criterion contained in a text segment, paragraph or sentence was marked with different colours based on the established descriptors. At this stage, information not related to the study was also eliminated.

c) Relational reading and axial coding

Once the open coding was completed, an analytical reading of the texts selected by color was carried out to identify patterns or similarities between the texts. A matrix was then drawn up with the criteria grouping the statements made by each participant at some point during the meeting, as shown in Table 11.

Table 11. Axial coding process and emerging categories

Number of texts	*Criteria*	*Participant*	*Emerging category*
T*(1,..,.n)*	There is a lack of knowledge about the disease.	*p1, p6,…,px*	Education and training
T*(1,...,n)*	It is necessary to educate the producer		
T*1,...,n)*	Farmers have not been adequately informed about the control plan.	*p6, p8, p14,…,px*	Promotion and communication

Tn: n reported texts; px: participant x

d) Selective coding

The patterns of information were analyzed and grouped inductively into new or emerging categories (Table 10).

2.5. Analysis conducted using the information collected in the focus groups of farmers

The information processed was used to assign a rating to four categories: level of knowledge of the disease, awareness and risk perception, risk practices and prevention.

The level of knowledge of bovine brucellosis among farmers was assessed through the following criteria: a) identification of bovine brucellosis either by name or by some local denomination, b) routes of transmission in animals or humans, c) problems caused by bovine brucellosis in cattle and humans, and d) identification of at least one form of prevention or control.

The level of awareness and risk perception were assessed according to the importance the farmers attached to the occurrence of bovine brucellosis, the actions taken to prevent its spread, and the measures implemented to prevent its introduction into the farm.

The rating of prevention measures was mainly related with the implementation of vaccination or testing to detect infected animals.

Risk practices were rated according to the number reported in each group. They were also listed and a rating was assigned to each one.

The following scale was used to rate the four categories described above and the risk practices: absence (-) in case of ignorance or non-reporting of an event by all participants; low (+) if an event was reported once; medium (++) in the case of an event reported by several participants; high (+++) in the case of an event that was reported by all the participants.

3. Results

3.1. Focus groups of veterinarians

The meetings of the focus groups of veterinarians lasted around 80 minutes. Seventeen criteria were identified which were grouped into four categories. The patterns of thinking according to these categories are summarized in Table 12.

i. Education on animal health and technical training

According to the participants, there is a low level of knowledge of brucellosis among farmers. Besides, those who have heard of the disease perceived the risk of brucellosis infection in both humans and animals as low.

> *"Farmers have very little knowledge of brucellosis, and those who know that it causes abortions in cows do not always understand that it is a zoonosis and even less how it is transmitted to humans."*

> *"Some farmers who have heard of brucellosis are unaware of the seriousness of the disease and keep positive animals on the farm or sell them."*

Table 12. Patterns of thinking of veterinarians on the issues of implementing the bovine brucellosis control plan in Ecuador

Category	*Criteria*
i. Education on animal health and technical training	• Low level of education on biosecurity in most of the farmers • Farmers do not know what bovine brucellosis is, its health implications and regulations • Low knowledge of good production, reproductive and biosecurity practices on farm. • Lack or poor record keeping • Technical training does not reach those in charge of the animals
ii. Promotion and communication of the National National Brucellosis Control Program (NBCP).	• Lack of knowledge of the existence or lack of awareness of the NBCP among farmers • Lack of interest and apathy for the participation of farmers in the NBCP • Farmers' fear of participating in the NBCP due to seizures and sanctions • Lack of confidence in brucellosis diagnostic and control procedures • Laboratory results provided to farmers after diagnosis have little credibility
iii. Actions of the official veterinary services	• Limitations of the official institution to enforce the law • The implementation of the control program does not consider preparing the farmer and explaining the deadlines for application of the law • Little knowledge of the actual brucellosis situation by zones • Weak information management and surveillance system for brucellosis • Scarce supply of laboratories for brucellosis diagnosis
iv. Incentives	• Incentives do not reach most farmers or in many cases do not compensate for the implementation of control measures • The lack of a compensation for brucellosis testing and vaccination discourages farmers' participation

The veterinarians also mentioned that there is a double lack of knowledge among farmers: they do not know what brucellosis is and they ignore the control regulations. These two factors and their low perception of the level of risk could be causing poor management of positive animals. It was mentioned that, most of the time, farmers keep suspect or positive animals on the farm, and in some cases, animals with a history of abortions are sold in markets.

The lack of technical training in animal health care, reproductive practices and record keeping would complicate the effectiveness of sanitary measures since many farmers and animal caretakers have precarious records or no records at all. Moreover, there is not a channel for farmers to informe them about the vaccine its use.

"In many cases, farmers need to be informed and made aware of the vaccine use because they do not have access to it or do not know how to handle it. As a result, they do not vaccinate even in endemic areas."

The participants agreed on the need to educate the farmers, differentiating education from technical training. Education involves awareness of the zoonotic risk of brucellosis and food hygiene. Technical training would be related to the transmission of technical knowledge on record management, sanitation, reproduction, diagnosis and prevention through vaccination.

Finally, they pointed out that training sessions are attended mainly by farm owners, but in very few cases by people in charge of the animals. This situation implies that the application of the control measures on the farms is not guaranteed. In rural indigenous territories the women are those who take care of the animals. In many cases, they only speak Kichwa and as the training sessions are not conducted in this language, they have to rely on the information provided by their husbands that have attended the training sessions instead of them.

"In some communities, mainly indigenous, training sessions are attended by men as heads of households. However, women are in charge of the animals in many places."

ii. Promotion and communication of the National Brucellosis Control Program (NBCP).

The control program promoted by the institution in charge of animal health was analyzed mainly by the private veterinary practitioners. They pointed out the lack of promotion of bovine brucellosis control strategies.

"Many farmers, mainly small producers, have yet to hear of the existence of the NBCP and those that have heard of, do not know or need to understand the benefits of their participation."

It was also mentioned that this plan does not favor farmers who deliver milk to intermediaries since it is not possible for them to receive the economic incentive that the dairy industry gives to brucellosis-free farm owners. This circumstance could generate a lack of interest and apathy to participate in the *NBCP*.

"*Some farmers know the plan but prefer not to participate for fear of confiscations. Also, in many cases, the compensation they receive for the milk does not represent the cost of the disposal of infected animals"*.

Participants mentioned, the distrust of farmers in laboratory results as a barrier to their participation in the control program.

"The way laboratory results are communicated could be more transparent. In addition, many producers need to rely more on laboratory technicians."

iii. Actions of the official veterinary services

Most of the veterinarians pointed out the need to strengthen the control institution to improve the efficiency of the intervention strategy and to enforce the law.

"*The farmer must be informed and given facilities and a deadline to apply the control measures. After the deadline, the law must be drastically enforced, even prohibiting the trade of animals without vaccination certificates."*

According to the participants, the disease is underdiagnosed due to the insufficient testing conducted in the farms. This situation would not give a true picture of the prevalence in many areas of the country. Likewise, the need to create a database to identify the brucellosis situation in each farm to implement adequate control measures was also mentioned. Although the budgetary and personnel limitations of the control institution throughout the country were acknowledged, accreditation of veterinarians and private laboratories are necessary.

With respect to disease control, it was proposed that dairy plants and dairies should play an active role in the brucellosis-free program. The context of this criterion is based on some experiences in the country, in which dairies or cheese production plants require guarantees of bovine brucellosis freedom from their suppliers. Additionally, it was mentioned that the control strategy should focus on brucellosis-free zones instead of certified brucellosis-free farms. Under this criterion, the main control strategy would be the vaccination and economic compensation would be applied to milk coming from a free zone.

iv. Incentives

In both focus groups of veterinarians it was mentioned the need to implement incentives and subsidies to reach most farmers, including those who deliver milk to intermediaries.

"*The cost of vaccines and diagnostic tests should be subsidized to encourage producer participation in the control program.*"

One participant pointed out the need to create a compensation or subsidy mechanism to cover the replacement of discarded cows, noting that this is the most critical loss for the producer.

"The farmer should be compensated for the culled animals, not necessarily with money, but by giving them young animals with good productive characteristics from brucellosis-free areas.".

Finally, several participants specified that the creation of private accredited laboratories should be encouraged through tax exemptions.

3.2. Focus groups of farmers

3.2.1. Description of the focus group of small-scale farmers

The two meetings with the farmers lasted about 45 minutes.

The focus group of small farmers was composed by 11 women and one man. The average age of the group was 56 year-old, the oldest attendee was a 67 year-old woman and the youngest was a 48 year old-woman. All of the attendees had only primary education and spoke Kichwa as their first language. All participants mentioned that their activities were dedicated to agriculture and animal husbandry (cattle, poultry and guinea pigs).

From the focus group discussion it was possible to establish that the presence of a majority of women was due to the migration of male members of the families (husbands or sons) to towns or the capital to work mainly in construction in towns, therefore, the women took care of the animals. Part of the income earned by the male members of the family was invested in animals.

"They (husbands) are also interested in the animals. Before, we did not even have a cow, and they worked elsewhere."

The only male participant mentioned:

"In the city, there is no work anymore, so I spend time with my wife at home taking care of the animals."

In all the farms, milking was done manually. Only three of the farms had breeding bulls. All of the participants mentioned selling milk to an intermediary (milkman), although the milk trade was dependent on the rainy season. Although having an almost permanent income from milk, the focus group participants did not identify cattle raising as their main livelihood.

"We don't have good dairy cows. We have ordinary cows and problems with the provision of grass.".

"When there is not enough grass for the cows, there is little milk, and the milkman does not come."

3.2.2. Description of the focus group of medium-scale farmers

The focus group of medium scale farmers consisted of eight attendees, mostly men, who belonged to a farmer association created two years earlier. The association manages a small milk collection center and a cheese processing plant. Milking was done mechanically on five farms and manually on three. All the farms had breeding bulls and in five farms, more than one bull. The producers delivered all their production to the association's collection center for industrialization and sale. Most of the farms had day laborers whose presence increased or decreased depending on the season.

3.2.3. Farm characteristics

The information on the productive characteristics of the farms collected through the survey at the end of the two meetings is summarized in Table 13.

Table 13. Characteristics of the cattle farms of the two groups of farmers

Farm characteristics	*Small farms*	*Medium farms*
Average farm size (h)	0.8 (0.5-2)	104 (45-220)
Average number of animals per farm	7 (5-11)	46 (18-75)
Average number of cows in production	4 (3-6)	23 (8-43)
Average milk production/cow/day (l)	4,5 (3-5.5)	6,4 (5-8)
Average liters of milk/farm/day (l)	18,6 (9-32)	140,6 (60-220)
Milk price	0,38	0,45
Average farm income/day (USd $)	8,4 (4.05- 14.4)	63 (27-99)
Milk destination	Collection center	Collection center

3.2.4. Criteria

The criteria were grouped around the defined categories. However, further to the analysis, additional categories emerged: 12 criteria were identified for the focus group of small scale-farmers and grouped into 5 categories and for the medium-scale farmers 15 criteria grouped into 4 categories (Table 14).

3.2.4.1. Small-scale farmers

i. Knowledge on bovine brucellosis

None of the participants knew about bovine brucellosis. They had never heard the term or knew that it was an abortive disease affecting cattle or that it was a zoonosis. However, when the clinical signs in cows were explained, two participants mentioned having had cases of abortions, placental retentions or increased days open. Some participants mentioned having had problems with abortions some time ago. However, they did not relate them to a specific disease.

"My calf died when it was about to come out."

"The cow aborted the first calf. Then she had another calf, and she recently accepted the bull again."

"This problem happened to me recently. The placenta did not come out of the cow."

Table 14. Thinking patterns on bovine brucellosis of farmers of the focus groups

	Criteria	
Category	*Small-scale farmers*	*Middle- scale farmers*
i. Knowledge on bovine brucellosis	They do not know or have never heard of the disease. They do not associate abortions with the possible presence of brucellosis.	They know that the disease exists but they do not know how it is transmitted. Abortions are explained by local beliefs.
ii. Awareness and risk perception	They do not consider the consumption of raw milk dangerous. They consume raw milk sporadically. They do not eliminate the aborted fetuses nor do they consider its consumption dangerous.	Those who have not witnessed abortions on the farm do not consider vaccination a priority. They do not consider dangerous to keep a cow that has aborted on the farm. They consider that cases of brucellosis in animals and only occur in particular situations.
iii. Risk practices	They move their animals for feeding or reproduction. They call any injectable product a vaccine. They do not request vaccination certificates against bovine brucellosis or brucellosis tests when marketing animals.	Keep cows that have aborted on the farm. Sick animals are not isolated. They do not apply sanitary procedures to eliminate aborted fetuses. They allow the dog access to aborted material. They do not know how to proceed in case of brucellosis positive tests. Cows that have aborted end up being sold. Milk is consumed on the farm or sold. They exchange bull without sanitary guarantees.
iv. Prevention	They are not aware of the existence of brucellosis vaccines.	Neither vaccination nor testing is widespread. Limited knowledge about the use of brucellosis vaccines.
v. Gender activity and need for technical assistance	Women need to receive technical assistance for animal care aid. Regarding animal raising, nobody takes women into account because they are women and for that reason they help each other.	

ii. Awareness and risk perception

When asked what they did with an aborted fetus, some participants said that they did not know what to do with it, and others mentioned different practices.

"I don't remember anymore. We cooked it, I think."

"It is left for the dogs to eat."

The participants mentioned the consumption of raw milk sporadically.

"I have taken the milk directly from the cow, but anything happened to me."

iii. Risk practices

When asked about the method they used for reproduction, 11 participants mentioned direct mating as the traditional method. When asked about how they organized the mating, three participants mentioned:

"*You have to look for someone who has a bull."*

"You have to take the cow where there is a bull."

"The neighbor's bull is rented."

These criteria indicate that, in all cases, reproduction was carried out by direct mounting and that most of the farmers did not have a breeding bull.

One participant mentioned having hired the services of an inseminator but acknowledged that it was not a common practice.

Most of the participants did not understand what a vaccine was. They made reference to an injectable product (antiparasitics or vitamins). Only two participants mentioned the vaccination against foot-and-mouth disease.

Regarding animal trade, the participants mentioned that there were not trade restrictions in the area. The history of the purchased animal is not usually investigated and the price is established according to the animal appearance.

"Here we trust the neighbors."

When asked where they purchased the animals and whether they requested vaccination certificates, they said:

"*I have brought the animals from Saquisilí (animal market). I only have the foot-and-mouth disease certificates. I don't know if other certificates exist.*"

or

"We buy and sell among ourselves."

The participants indicated moving the animals in times of drought and scarcity of grass in the area.

"*When it does not rain, we have to find grass and take the animals there.*"

"The grass is rented, and the cow is left tied or loose."

iv. Prevention

The farmers did not know about prevention mechanisms, nor did they understand that the vaccine protects, and even less that vaccines exist to prevent brucellosis.

v. Gender activity and need for technical assistance

The participants talked on several occasions about the participation of women and the need to receive technical assistance in animal care. They also indicated that the women of the gathered group consulted and helped each other, but acknowledged that they did not have lacked basic knowledge of animal health.

"None of us knows how to cure the cows, and sometimes there is no one to help us. It would be good if someone could teach us."

"In my case, I did not know how to treat my cow, so I waited until she gave birth and sold her."

It was also mentioned that, on certain occasions, "people" from the neighboring town came to administer deworming, supply vitamins or carry out private insemination. It was pointed out that there was already a veterinarian in the neighboring town, and that the veterinarian's services were only used in emergencies.

"We look for the vet when there are emergencies, like when the cow cannot give birth."

3.2.4.2. Medium-scale farmers

i. Brucellosis knowledge

This group of farmers knew that bovine brucellosis affect cattle and that it could occur even in humans. However, they were not aware about its routes of transmission or the prevention measures to be applied on the farm.

During the meeting, it was mentioned that abortions were recurrent and that they were a problem for the farmers. Three of the attendees mentioned having had late-gestation abortions on their farms. They pointed out that abortions in cows can occur for multiple reasons. One participant attributed cases of abortion to plant consumption and animal behavior:

"Since the time of our grandparents, we've known that the cows mainly abort when they eat a certain plant. Sometimes, cows also hurt each other before giving birth, which could cause abortion."

One of the participants asked if brucellosis could occur in other animals.

ii. Awareness and risk perception

Different criteria emerged on the risk of presence and contagion of the disease in animals. Two farmers who have had abortions on their farms have already applied the vaccine. The other farmers did not consider vaccination to be essential. It was also mentioned that even in the event of abortions, the animals could remain on the farm until the end of lactation, although it was not specified whether they were isolated.

"I had a cow that aborted only once, and from then on, it remained normal."

"In my case, the cow aborted days before calving. However, the cow had milk and could not her from the herd."

The group knew that people could become infected by brucellosis but did not understand how. One of the attendees reported that he had been infected with brucellosis and hospitalized for more than two months. The other attendees knew that this farmer had been ill, but did not know it was due to brucellosis. The story helped them to relate the case to the danger of the disease.

"I went to help a first-calving cow that could not give birth, and when I cut her, the calf fell on top of me with all the cow's fluids, and I had nothing to clean myself with. So this is how I got infected. All I got was chills, my joints did not hurt, but my back did. While working, I had no

pain but I had to lie down in the afternoon. I was short of breath, some spots came out, and I left. I took some tests, and they told me it was brucellosis. I was in a clinic for two months."

The group of farmers had a distorted perception of a zoonotic risk, although one infected person was among the attendees.

None of the attendees mentioned the existence of regulations or knew about the brucellosis-free-farm program.

iii. Risk practices

When it was asked what they did in the event of abortion three different criteria were obtained, two of which were risk practices.

"Abortion remains are buried."

"We do not bury them, we leave them lying around."

"Then the dogs or the vultures eat them and leave the land clean."

It was explained to the participants that dogs could be reservoirs of the disease. Two of the participants mentioned:

"*I have forbidden dogs from entering (the pastures)."*

The farmer who was infected with brucellosis said:

"Furthermore, I have eliminated dogs, chickens and pigs to avoid them being in contact with the farm."

Regarding the fate of cows with a history of abortion many farmers kept the cow on the farm to take advantage of milk production.

"Depending on the cow, you don't sell it if it's a good cow."

Two criteria emerged from this answer: if the animal is in good condition, it will remain on the farm; a cow potentially infected with brucellosis can be sold.

Although the farmers had an idea of the implications of the occurrence of bovine brucellosis on the farm, most participants were unaware of the measures applied in the event of a positive result. For example, when it was mentioned that an animal testing positive for brucellosis should be eliminated, another participant pointed out:

"The farmer will not slaughter the cow if it produces milk or is fat (in good condition)."

The criterion of keeping cows in the knowledge that they may be infected was confirmed by the answers to the question about using the milk if the cow was infected with bovine brucellosis. The attendees answered:

"The milk is sold or we drink it ourselves."

"It can also happen that the cow is ready to give birth, so the milk is used until the cow dries up."

Although several cases of brucellosis infection in humans were known in the area, most assumed they were extraordinary events like the one reported by one of the attendees. They did not associate the infection with the consumption of unpasteurized dairy products.

Regarding reproductive management, all participants mentioned having a breeding bull. In addition, the rent, loan or exchange of the bulls seemed to be a general practice.

"As they are friends or sometimes family members, we lend them the bull without knowing whether the cow is healthy or sick."

However, mating is not always supervised by the farmer. The lack of adequate fencing allows bulls to enter neighboring farms.

"Sometimes the cow or bull moves on to other farms."

"The neighbors take advantage of my bull for their cows."

iv. Prevention

Regarding vaccination as a preventive measure, only two of the attendees said that they vaccinated against brucellosis. One of these farmers did so after several abortions of his cows. The same farmer did frequent testing to know the status of brucellosis on his farm. The second one was the one that was infected with brucellosis. The other assistants did not consider it as essential, as the most obvious problems were related to other diseases. In addition, they did not know the vaccination procedure and the types of vaccine.

"I was forced to have tests done on my cows and had to cull 4-5 cows."

"If one cow turns out to be infected, do I still have to vaccinate the others?"

When asked if farmers required a certificate of vaccination or a guarantee that the animal was free of brucellosis at the time of purchase, most of the attendees answered that they did not.

"I'm going to tell you the truth, no."

"No, usually, we just buy."

"For us, it is enough when we are told the cow produces 8-9 liters of milk. If so, we buy it."

3.2.5. Rating for items related with bovine brucellosis knowledge/awareness and risk practices

The group of small-scale farmers showed lower rating for items related with bovine brucellosis knowledge/awareness and risk practices than the group of medium-scale farmers, except for risk practices (Table 15).

Table 15. Rating for knowledge about bovine brucellosis, awareness, and risk practices in the focus group of farmers

Category	*Small-scale farmers*	*Medium-scale farmers*
Knowledge on bovine brucelosis	−	+
Level of awareness and risk perception	−	+
Risk practices	++	++
Prevention	−	+

Scale: Absent (−), Low (+), Medium (++), High (+++)

Risk practices were reported by both groups, mainly regarding the trade of suspicious animals, the exchange of bulls and the maintenance of animals with a history of abortion on the farms to take advantage of milk production (Table 16).

Table 16. Rating for the risk practices reported in the focus groups of farmers

Risk practice	*Small-scale farmers*	*Medium-scale farmers*
Consumption of raw milk	++	−
Consumption of milk from suspect animals	+	+++
Consumption and handling of abortions	++	−
Leaving abortions out in the open	−	++
Keeping sick animals with a history of abortion on the farm	+	+++
Moving animals to other farms	++	++
Exchange breeding bulls without health certificates	+++	+++
Commercialize animals without sanitary guarantee	+++	+++

Scale: Absent (−), Low (+), Medium (++), High (+++)

4. Discussion

We studied the perception of the problem of bovine brucellosis in Ecuador in a focus group of veterinarians implied in cattle health and inquired about the level of knowledge, awareness and risk perception of the presence of brucellosis in two focus groups of small- and medium-scale farmers, using the qualitative technique of data collection and analysis.

In the focus group of veterinarians, four categories linked with two axes were identified: the role of the farmer in the control of the disease, and the actions carried out by the animal health control institution. The criteria related with the lack of knowledge on brucellosis, training and awareness can be crosschecked with the related categories of the two groups of farmers.

Regarding knowledge, the indigenous group clearly stated that they did not know about the disease. As for the medium-scale farmers they knew the name of the disease but not the transmission routes or the measures to apply in case of detection. We can therefore infer that the lack of knowledge is an important issue among small- and medium-scale farmers. The lack of knowledge on the disease among the indigenous people was reported in a survey conducted in 71 small scale farmers belonging to two indigenous communities in the Andean province of Chimborazo. It was found a complete lack of knowledge on brucellosis (Uvidia et al., 2018). There are some factors that may explain the poor level of knowledge on brucellosis or the non-access to technical training in these indigenous communities that were identified in the indigenous community focal group: the low level of education, age, language barriers (main language spoken is Kichwa) and gender.

In our study, the focus group of indigenous was almost composed by women who were in charge of the care of the animals due to the absence of their husbands or sons working in towns or cities. The women mentioned not having access to training and as a consequence, they did not know how to take care of animals. This gender issue seems not to be only limited to indigenous communities. In one of the focus group of veterinarians, a participant that provided technical assistance in the field, made reference to the fact that trainings were attended only by men as heads of household, while women were in charge of the animals. According to the FAO (FAO, 2011), the presence of women in agricultural and livestock activities is increasing worldwide and they are systematically discriminated in terms of access to the resources needed for agricultural production and socio-economic development.

The women in the focus group of indigenous farmers had an average age of 48 year-old. The age of the farmers is a factor that must be considered in any strategy that is carried out with small farmers in the rural sector, since it has been reported that in the older population there is also a high level of illiteracy or low schooling. In addition, over time they stop taking part in community activities especially if they are women (Waters & Gallegos, C, 2014). It has been estimated that in Ecuador, out of 842,882 farmers, 13.9% (117,243) are indigenous and 24.5% (213,731) are women. Of this total of women farmers 27.7% (59,203) are over 61 years; 28.4% (65,873) are illiterate, and 59.5% (127,184) have only primary education (INEC, 2008). The conjunction of these characteristics could be restricting the access of numerous groups of farmers, mainly women, to available health information, which has not been designed for these populations. Moreover, their productivity is not visible and they are generally unpaid in poor rural sectors (Herrera et al., 2017). Different studies have pointed out that the work and role of women in the Ecuadorian rural sector is perceived as only relevant in the domestic sphere and that women's access to training in cattle raising may be stigmatized by men (Flores & Sigcha, 2018; Ramirez et al., 2019).

The medium-scale farmers from the focus group of Manabí province ignored the bovine brucellosis transmission routes, the measures to apply in case of its detection and its potential transmission to humans. A lack of knowledge on brucellosis had already been reported in 95% of the farmers surveyed in a study conducted in the same province (Zambrano Aguayo et al., 2016). In a survey conducted as well in Manabí province in people working in the bovine production chain, 117 surveyed farmers out of 156 (75%) were unaware of bovine brucellosis (Pérez Ruano & Aguayo, 2017). In both studies, it is specified that despite that lack of knowledge, some of those farmers applied the vaccination. This was also the case among some participants of the focus group. It is possible that vaccination against brucellosis is encouraged by dairy plants or dairies for milk to which the farmers sell the milk produced in their farms.

A low level of awareness about the presence of the disease and the low risk perception of farmers could be influencing the health status of the disease in many areas. Certain practices, as well, could increase the direct or indirect exposure to brucellosis among humans and animals, favoring its presence and spread (Mantur & Amarnath, 2008; Mendoza de Arbo et al., 2018). Risk practices that were mentioned in the focus groups of veterinarians and farmers are potential risk factors for the occurrence of brucellosis in humans and animals, such as the lack of vaccination in high prevalence areas, trade and exchange of animals without health certificates, keeping suspect or sick animals on farms, aborted material left in the open field, or consumption of raw milk or aborted fetuses. These practices have also been identified as risk factors in studies conducted in some provinces of the coast and the Andean region. (Carbonero et al., 2018; Maniato E. et al., 2022; Zambrano Aguayo et al., 2016).

In Ecuador, there is very little information available on the connection between the risk of brucellosis infection in cattle and the lack of training and education of cattle farmers (Díez & Coelho, 2013). The issue of training and health education was one of the categories to which several criteria discussed by the focus group of veterinarians converged. They emphasized, however, that training and education are not the same thing. Training would consist in the transfer of technologies, while health education would include sensitizing the farmer to increase the level of awareness of the problems so that knowledge of animal management can be applied. Some authors have pointed out that ignorance of the disease would prevent identifying clinical signs compatible with brucellosis and, therefore, the lack of application of control measures. In this sense, knowledge of bovine brucellosis among farmers is a decisive factor in the successful implementation of sanitary measures to control and eradicate the disease (Adesokan et al., 2013; Kansiime et al., 2014; Mendoza de Arbo et al., 2018).

According to veterinarians from the focus groups, insufficient knowledge of bovine brucellosis, lack of interest, fear of sanctions, and lack of compensation mechanisms for the elimination of animals could limit the participation in the official bovine brucellosis control program that has been implemented on a voluntary basis. Besides, there has been weak institutional presence in the control and lack of communication and promotion strategy for the program. These factors could explain the endemicity of the disease.

Lack of interest and apathy of farmers have also been reported in farmers engaged in the official bovine brucellosis control program. In a study conducted in the Manabí province aiming to evaluate the application of procedures among farmers participating in the control program, a low level of compliance and even desertions were found. In addition, it was determined that monitoring measures are not complied with and there is no training for farmers (Zambrano Aguayo & Pérez Ruano, 2016).

The participants of the focus group of veterinarians mentioned that the milk and cheese processing industries should be included in a strategy of bovine brucellosis control through the requirement of guarantees of bovine brucellosis freedom from their suppliers. In this regard, it is important to note that the brucellosis free farm program has been successfully implemented when it has been supported by farmers' associations or private enterprises. Such support has also contributed to the farmer awareness. In a study involving farmer suppliers of an Ecuadorian milk processing company, 82% of the farmers knew about the mechanisms of the transmission of the disease, the clinical signs of brucellosis and the risks involved in consuming unpasteurized products (Cadena, 2019).

5. Conclusion

The focus group methodology allowed us to identify several social aspects involved in the bovine brucellosis situation in Ecuador. As the eradication of bovine brucellosis is multifactorial, the success of the national eradication program cannot be based on technical aspects alone but should also address the social aspects. Training campaigns for farmers should be provided ensuring the participation of both genders. Some type of benefit for farmers participating in the bovine brucellosis control program in economically disadvantaged areas could be envisaged.

GENERAL DISCUSSION

The main objectives of my PhD work were to study the possible implication of the cattle trade network in the spread of bovine brucellosis in Ecuador and the role played by small- and medium-scale cattle farmers in the maintenance of the disease.

The construction of the cattle trade networks began with the processing of two databases provided by the Ecuadorian veterinary services. The first database included the national census of cattle farms. It contained information on the location of the farms, specifying their administrative units (province, canton and parish), geographical coordinates, type of livestock (meat, milk, mixed) and type of cattle (calf, heifer, cow, bull). The second database contained the cattle movements with records of the date of movement, cattle owner's name and identity card number, a certified code delivered by the veterinary services, and origin/destination specifying the holding name, parish, canton and province. The RStudio software (R Core Team 2021) was very useful in processing the information to achieve error-free databases. However, in the process, a large amount of data had to be deleted due to different factors. In both databases, data were repeated, names of the same cattle owner were entered in different ways or locations, data rows were incomplete, or identity numbers were wrong. When attempting to correct records according to locations, an additional difficult was that a parish name could be found in different cantons or provinces. We crossed both databases to use the location coordinates and the type of farm that were lacking in the movement database but it was not possible due to inconsistencies in the owner’s name or identification. In addition, there was a large amount of missing data on coordinates in the cattle census database. During the data cleansing process, we contacted the people in charge of the databases several times who explained that some of the errors were associated with the fact that the information still needed to be manually entered in some zones of the country. Regarding the cattle movement database, some movements were linked to people without any farm record that moved animals from farms to markets or from markets to other markets. These traders were not clearly identified as such in the database.

Our initial purpose was to perform an analysis at the farm level. However, after examining the databases, we conducted the study at the parish level since we found inconsistencies between the two types of bases. Therefore, it is possible that a network analysis at the holding (farm/market) scale would have yielded different results. In this sense, it is essential to draw the attention of the national veterinary services to improve the information collection, storage and management system to avoid as much as possible failures in the records and to take advantage of the national databases as analysis resources for surveillance and design of control plans.

We built a network that included all the traded cattle to consider the potential spread of a rapidly spreading pathogen such as an FMD virus from a different serotype not included in the FMD vaccine currently used. We could identify the high centrality that the parishes with markets represented within the network, since half of the cattle movements and transfers originating from farms were directed to them. In addition, parishes with markets were highly connected with parishes without markets to which cattle arrived even from distant areas of the country, hence the negative values of assortativity. In an FMD introduction scenario, the parishes where the markets are located would be the places where surveillance interventions should be reinforced, or that should be closed to stop the spread of this disease.

We also built a network of only cows to consider the transmission of bovine brucellosis. The analysis of that network at the parish level made it possible to identify communities that corresponded to regions where cow trade was most intense. The identification of these regions could constitute a resource for the design and implementation of control plans, based on brucellosis-free zones with vaccination, as suggested in the focus group of veterinarians.

Regarding the level of diffusion of the two diseases, both the all cattle network and the cow network had large strong components that included more than 90% of the nodes, which means that both diseases could reach practically the entire national territory through the movement of infected animals. Furthermore, characteristics of small-world networks were identified in both cases, meaning that an infection could spread locally among neighboring parishes but also jump to distant sites. Unlike the controls currently applied to FMD, which include compulsory vaccination and control of the vaccination of the moved cattle, in the case of bovine brucellosis, neither of these requirements has been implemented.

The future studies to conduct on the cattle movements should be based on more accurate data. In this sense, the two databases available in the veterinary services need to be improved. The census database could include information on different categories of farms according to the purpose and the size of the farm. This information could also be available in the cattle movement database. In the frame of the control of bovine brucellosis, the farms that are participating in the farm bovine brucellosis free program could also be identified on an annual basis. Information on cattle movements should be automated as much as possible to avoid mistakes. The inconsistencies of the databases identified in our work will be reported to the official veterinary services.

To address the lack of information on bovine brucellosis in small and medium farms, we used two tools: a risk factor survey analysis to identify factors associated with the presence of the disease, and a qualitative study using a focus group of small-scale farmers and a focus group of middle-scale farmers to establish their level of knowledge, awareness and perception of risk in relation to bovine brucellosis.

We considered essential to conduct the risk factor study in a region with a high prevalence of brucellosis, where no studies had been conducted previously. We found that medium-sized farms had significantly higher prevalence values and higher odds of becoming infected than small farms due to the farm size. Reproductive problems were also more prevalent in this farm group. Although both types of farmers were at risk, it should be considered that the medium-scale farmers carried out comparatively more commercial transactions and move a greater number of animals to and from their farms, thus increasing the probability of exchanging infected animals. The medium-scale farmers could be in a process of transition to a commercial scheme that is reflected by the increase in the size of their herds and in the frequency of commercial exchanges of animals. These changes apparently do not go at the same speed as the training in reproductive and health management that could allow them to face the challenges related with bovine brucellosis.

The accessibility to accurate information may have biased some data of this second study, mainly from the medium farms. First, the owner was not always present on some farms, and those who answered the questions were employees who, in some cases, were temporary workers who did not have all the information regarding the farm, such as records on abortions or vaccination. Second, it is possible that some medium-scale farmers who had heard about brucellosis were reluctant to provide reliable information, mainly about the vaccination implementation or the occurrence of abortions for fear of sanctions by the control institution, such as the confiscation of cattle.

The qualitative analysis (focus group technique) allowed us to identify issues that could only be highlighted with this technique, which shows that this tool should be used more frequently when it comes to epidemiological studies in order to have a complete picture of a situation. We identified that small-scale farmers did not have access to information on bovine brucellosis and that they ignored the strategies currently used to control the disease. Their awareness level and perception of risk were low. This led them to carry out risk practices that could allow the transmission of bovine brucellosis to animals or humans. Some factors identified as risk practices in the Quinindé study, such as the consumption of raw milk or the consumption of aborted fetus, were also indicated in the focus groups of veterinarians and farmers.

The low level of knowledge in the small- and medium-scale farmers was pointed out by the participants of the focus groups of veterinarians and is in accordance with the findings obtained in the focus groups of farmers. The low level of knowledge of the disease in the two types of farmers could be contributing to the persistence of the disease. Farmer education and awareness could help improve the effectiveness of the brucellosis control plan and its sustainability, as suggested by the participants in the farmer focus groups.

Another important factor identified in the focus groups of farmers, and that had not been considered in epidemiological studies linked to cattle farming in Ecuador, was the situation of women farmers. The education of women working in agriculture should be encouraged by the national authorities.

We could also identify that the medium- and mainly the small-scale farmers did not have the same opportunity to receive a compensation if their farms were brucellosis-free as the commercial farmers, because they marketed their products through intermediaries. However, there have been successful initiatives in Ecuador, where the diary industries have encouraged brucellosis control by purchasing milk from small- or medium-sized farms belonging to farmer associations. To understand the mechanisms that could be applied to achieve the participation in the bovine brucellosis control program, we have launched focus groups including commercial cattle farmers, dairy industry managers and small- and medium-scale farmers that currently sell to processing plants.

Several factors should be considered when conducting fieldworks with small- and medium-scale farmers. First, small-scale farmers can be reluctant to share information with the researchers because of their distrust of city-dwellers. The presence of a community leader could help secure their collaboration. Second, participatory techniques should be used to get all the farmers to interact in order to avoid the participation of a few attendees. In the case of focus groups meetings, the capture of information should be supported by several recordings, notes, or other supporting media since, sometimes, the records can be difficult to hear or incomprehensible, causing valuable information to be lost. Finally, recordings must be discreet, as they can intimidate the attendees.

The brucellosis control program launched in 2010 included, in addition to technical strategies such as screening and vaccination, the implementation of education and awareness campaigns aimed at farmers, as well as information programs via the media. From the results obtained in the studies that we conducted in the small and medium-scale farmers, it is clear that education and awareness campaigns are essential aspects to be included and carried out in the current bovine brucellosis control strategies in order to make the control plan more efficient and sustainable.

CONCLUSION

Bovine brucellosis in Ecuador is a multifactorial health problem that include multiple aspects that should be addressed such as cattle trade, human activities related to cattle management, and sociocultural factors.

The epidemiology methods and sociological analysis used during my PhD have contributed to a better understanding of the bovine brucellosis situation in Ecuador through the analysis of cattle movements, the identification of risk factors in small- and medium-sized farms, and the assessment of their level of knowledge and awareness of the disease. We hope that the results obtained will be helpful for the Ecuadorian veterinary services and farmers.

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APPENDIX

1. Questionnaire applied in the risk factor study

Ubicación

Provincia de Esmeraldas, Cantón: Quinindé, Parroquia:____
Sector o Recinto:____
Nombre de la Finca______________________________________

La Finca

1. ¿La principal actividad de la finca es?: Agricultura____ Ganadería_____
2. Quien maneja la Finca es: Propietario [___], Encargado [___]
3. Pertenece a alguna asociación de productores? si [__], no [__]
4. Lleva algún tipo de registro de Ganadería? si [__], no [__]
5. Recibe asistencia técnica? si [__], no [__] de quien: MAGAP [__] Agrocalidad [__] Casas comerciales[__]
6. Quién pasa con el ganado: Familia [___], jornaleros [___], Vecinos [___] Otros [__].
   Familiares (Cuales) Hijos[__], Esposa[__], otros_______________
7. Cuáles de las siguientes instalaciones posee la finca (***solo marque lo que existe***): Potreros separados [__], Manga o Embudo [__], zona de ordeño [__], ordeño mecánico[__] bodega [__]
8. Cuantas hectáreas de potrero hay en la finca______ Cual es el área de la finca?_______
9. Cuáles de los siguientes servicios tiene la finca: Luz eléctrica [__] Agua entubada [__], internet [__], televisión por cable [__]
10. En caso de emergencias veterinarias a quien pide ayuda: Vecinos[__]MAGAP[__] Agrocalidad [__] Otros (quien)__________________, ninguno [__]

Estructura del Hato

11. Cuál es la raza de ganado que predomina en la finca? [____]
12. Cuantos animales existen al momento en el hato? Vacas en producción [________], Vientres [______] Vaconas [___], Toretes [____], Terneros [____] Machos reproductores [______]
13. Cuantos animales ha introducido este año?____(Motivo): Engorde[__]Aumento del hato[__] Reproductor [__] Mejoramiento raza [__] Otro__________________
14. ¿Si una enfermedad se presenta donde un vecino, podría llegar a su finca? si [__], no [__]
15. Las labores ganaderas que realiza con el ganado son: Desparasitación [__], Vitaminas y suplementos [__], Vacunación [__]
16. Qué vacunas? Aftosa [__] triple (*clostridiales*) [__] **Brucelosis**[__] RB51 [__] Cepa 19 [__] otra______
17. En los últimos 2 años se han registrado abortos en la finca? si [__], no [__]
18. En los últimos 2 años han nacido terneros muertos? si [__], no [__]
19. Existen vacas infértiles? si [__], no [__]
20. Las vacas paren en: Establo[__] a campo abierto[__] Otro______________
21. Posee otros animales perros (número)[__] caballos [__] mulares [__] cerdos[__]gatos[__], Cabras[__] Ovejas[__]

Rastreo

22. Donde compra sus animales: Feria [__] vecinos [__] nacen en finca[__] otro: __________________
23. Cuando Ud., tiene forraje, alquila sus potreros para animales de otras fincas? si [__], no [__]
24. Cuando usted necesita forraje **lleva** a sus animales a potreros de alquiler: si [__], no [__]
25. El agua **que toma** su ganado pasa por otras fincas: si [__], no [__]
26. Los puntos donde toman agua sus animales se comparten con otras fincas si [__], no [__]
27. Ha llevado a sus animales a ferias ganaderas o concursos? si [__], no [__]
28. La reproducción del ganado la realiza por: **Monta** [__] Controlada (si/no) [__] Inseminación [__].
29. Si se realiza por monta: Alquila toro reproductor [__] Tiene toro reproductor propio [__]
30. Si la finca posee reproductor lo alquila para servicio? si [__], no [__]
31. Sus animales son vendidos en: Feria [__] a vecinos [__] a comerciantes [__] Otro

Trazabilidad	32. Sus animales son vendidos en: Feria [__] a vecinos [__] a comerciantes [__] Otro______________________ 33. Los productos que salen de la finca: **Leche [__]:** Permanente [__]; temporadas[__]; Eventualmente [__] La leche de la finca se vende principalmente en: ________________________ 34. Cuantos litros de leche produce al momento [_____], Litros promedio [____] 35. **Produce Queso** No [__], **SI** [___]: Venta[__] o consumo [__] 36. Con que frecuencia saca el queso: Diario [__], Semanal [__],Quincenal [__], Mensual[__], Rara vez [__] 37. Quien hace el Queso: Esposa [__] Esposo [__] Otro_______________ 38. Los utensilios que utiliza son: de la casa [___] comprados solo para el queso [___] 39. El queso se vende a: Comerciantes [___] Directo en el poblado [__] feria [___] Otro____ 40. Comparte utensilios como de ganadería con otras fincas? Si [__], no [__] 41. Ud. o su familia consume estos productos?: Leche cruda, [__] Queso fresco, [__]Cuajada[__]Ninguno [__]
Abortos	42. Si se produce un aborto que hace con los restos?: entierra [___] incinera [___] se quedan en el potrero [__] les da a los perros [___], se hace Chivo hornado [___] otro____________ 43. ¿Qué hace con la vaca que aborta? Se queda en la finca hasta que se recupere[__] Se la vende[__] Se envía a la faena [__] otro____
Brucelosis	44. Conoce que es la brucelosis y como se transmite? si [__], no [__] 45. Sabe si la brucelosis es pasosa a los humanos? si [__], no [__] 46. En los últimos 5 años, alguien de la familia ha sido diagnosticado con brucelosis?: si [__], no[__] 47. ¿Ha oído del programa de fincas libres a Brucelosis y Tuberculosis de AGROCALIDAD?: si [__], no [__] 48. (***Si la respuesta anterior es sí***) Le interesaría participar en el programa? si [__], no [__] 49. (***Si la respuesta anterior es no***) Por qué no participaría? Costoso [__] No es importante [__] Es muy complicado [__] Es más para fincas grandes [__] Otro____________________
Desechos	50. ¿Qué hace con el estiércol de los establos?: Lo deja en el establo [__] Lo usa como abono[__] se lo desecha en la quebrada o estero [__] 51. ¿Qué hace con los frascos vacíos de medicamentos?: Basura común [__] Quema [__] Quebrada[__]
Productor	52. Asiste a capacitaciones de ganadería: SI____ NO____ 53. A qué tipo de capacitaciones asiste: Charlas [__] Cursos [__]Seminarios[__],Otro____ 54. Se dedica a otras actividades laborales adicionales? Cuales?____________________________ 55. ¿Se informa de temas ganaderos por medio de?: Vecinos [__] Radio [__] TV[__] Internet [__] Ferias [__] Otros____ 56. Como se moviliza: Transporte público/alquiler [__] moto [__] vehículo propio [__]Mulas o caballo[__] 57. Cuál fue su último grado de instrucción del encargado de la Finca?: Escuela[__] Colegio[__] Universidad[__] **Coordenadas: X [ ], Y:[ ]**