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Abstract

In recent decades, remote sensing (RS) technology and geographical information systems (GIS) were increasingly used as tools for epidemiological studies and the control of zoonotic diseases. Fasciolosis, a zoonotic disease caused by a trematode parasite (*Fasciola* spp.), is a good candidate for the application of RS and GIS in epidemiology because it is strongly influenced by the environment, i.e. the habitat of the intermediate host. In this study, we examined variables which may increase the fasciolosis risk of Ankole cattle in the degraded and overgrazed Mutara rangelands of north-eastern Rwanda. The risk variables considered included three environmental variables (normalized difference vegetation index, NDVI; normalized difference moisture index, NDMI; normalized difference water index, NDWI), two landscape metric variables (rangeland proportion, building density), two geological variables (poorly-drained soil proportion, elevation) and three animal husbandry variables (herb size, adult proportion and the body condition score). *Fasciola* spp. prevalence was used as the dependent variable, sampling season as a fixed factor and four principal components (PCs, condensed from the ten risk variables) as covariates in a univariate General Linear Model. *Fasciola* spp. prevalence was positively correlated to rangeland proportion, cattle herd size in rural areas, adult proportion and individual body condition. Moreover, high *Fasciola* spp. prevalence was found in densely vegetated areas with high moisture (high values of NDVI and NDMI), in combination with large proportions of poorly-drained soil at low elevations. Future investigations should focus on increased sampling across the Mutara rangelands to prepare a predictive, spatial fasciolosis risk map that would help to further improve sustainable land-use management.

Key words: *Fasciola*, Geographic Information System, Remote sensing, risk model, cattle husbandry, environmental factors, Rwanda

1. Introduction

During the past few decades, multi-disciplinary approaches to carry out epidemiology studies using remote sensing (RS) and geographical information systems (GIS) have been extensively applied (Hay, 2000; Thomson and Conner, 2000; Hendrickx et al., 2004; Kitron et al., 2006). Advances in RS have provided the ability to obtain a variety of environmental parameters (e.g. normalized difference vegetation index, NDVI; normalized difference moisture index, NDMI; normalized difference water index, NDWI) with numerous spatial and temporal resolutions that can be related to disease outbreaks and vector distribution (Hay et al., 1997; Robinson, 2000). GIS allows computer-based analysis of multiple layers of digital mapped data, such as satellite sensor data, maps of host populations, vector and disease distributions (Malone et al., 1997; Malone and Yilma, 1999). For example, environmental RS indices and GIS technologies have been applied to identify habitats of parasites and their vectors, such as mosquito-borne diseases (e.g. malaria, Rift Valley fever and dengue); snail-borne diseases (e.g. schistosomiasis and fasciolosis); or tick-borne diseases (e.g. boreliosis; Hay, 2000; Omumbo et al., 2002; Hendrickx et al., 2004; Tatem et al., 2004; Charlier et al., 2014).

Bovine fasciolosis is a zoonotic disease affecting the liver of wild and domestic ruminants, caused by parasitic trematodes of the genus *Fasciola (F. hepatica* or *F. gigantica*). The adult parasite lives in the bile ducts of the hosts' liver and causes substantial financial losses to pastoralist communities worldwide by negatively affecting growth rates and productive parameters (McCann et al., 2010; Byrne et al., 2016). In the early 2000s, global economic losses due to fasciolosis exceeded US$200 million, with about 300 million cattle infected (Mas-Coma et al., 2005; Dutra et al., 2010). Moreover, owing to the fact that *Fasciola* spp. also infect humans (currently 2.4 to 17 million people are infected with *F. hepatica*; Eslami et al., 2009), fasciolosis represents a serious public health threat, especially in developing countries (Rokni et al., 2002; Mas-Coma et al., 2009). The parasite occurs primarily in swampy areas or on flooded pastures, i.e. the preferred habitat of the intermediate host (pulmonate freshwater gastropods of the family Lymnaeidae; Brown, 2005; Torgerson and Claxton, 1999). Bovine livestock usually become infected by eating water plants and grass from inundated lawns, or simply by drinking contaminated water (Witenberg, 1964). Since the parasite is strongly influenced by the environment, i.e. the habitat of the intermediate host and by the relative longevity of the parasite inside the mammalian host, fasciolosis is an ideal candidate for the application of RS and GIS (Malone and Yilma, 1999).

Thus, the purpose of this study was to generate a risk model to better understand the epidemiology of *Fasciola* spp. in the degraded and overgrazed Mutara rangelands of north-eastern Rwanda. Hereby, we considered it imperative to follow a holistic approach and to relate parasite data to three environmental variables (NDVI, NDMI and NDWI), two landscape metric variables (rangeland proportion and building density), two geological variables (proportion of poorly-drained soil and elevation) and three animal husbandry variables (herd size, adult proportion and body condition score, BCS) to identify the major risk factors of *Fasciola* spp. infection in Ankole cattle.

2. Material and Methods

2.1 Study area

The Mutara rangelands are located in the Nyagatare District in north-eastern Rwanda (Fig. 1). They are characterised by a tropical rainfall pattern (wet season: March to May and October to November) with an average annual precipitation of 827 mm and a mean annual ambient temperature of 26.5°C. The Mutara rangelands comprise vast open grasslands, interspersed by evergreen bushland and thicket (Kindt et al., 2014) and are traditionally used to graze cattle. Today, the Mutara rangelands harbour an estimated 160,000 cattle, resulting in a cattle density of 81 individuals/km^2 (Wronski et al., 2017). Moreover, in a significant part of the Mutara, increasing subsistence agriculture and urbanization, leaving only 13% of the total land area in a natural state (CIRAD, 2002; Wronski et al., 2017).

2.2 Study animals and faecal sampling

Faecal samples were taken from Ankole cattle, a breed derived from the Sanga type cattle predominantly found in East-Central Africa (Epstein, 1957). Faecal samples were collected at the end of the short and long wet season, i.e. from 19 February to 17 March and from 12 June to 17 July 2016, respectively. In total, 570 faecal samples were obtained from 142 cattle herds. Sampled individuals were randomly encountered along three 2.5 km wide transect belts (22.5, 32.5 and 37.5 km long) stretching between the Tanzanian border (or the border of the modern Akagera NP) in the East and the Ugandan border (or the Byumba Escarpment) in the West (Fig. 1). Three to five faecal samples were collected from each herd directly after a focal animal had defecated. Additionally, coordinates were recorded using an Etrex 20x GPS (Garmin, USA). Faecal samples (30 g/individual) were retained in labelled plastic containers and preserved in 5-10% formalin prior to processing in the laboratory. Since most herds comprised of only females and their offspring (bulls are usually kept in the kraal), only females and their calves were sampled. Exotic Friesians (or hybrids with Ankole cattle), individuals treated with flukicides during the last six months prior to sampling, or individuals that did not experience similar husbandry conditions (e.g. overnight kraaling) were also excluded from our sampling. Moreover, date, time, age composition (number of adults and juveniles), herd size and the BCS of each sampled individual were recorded.

2.3 Coprological examination

Faecal samples were processed in the Veterinary Laboratory of Nyagatare Campus, University of Rwanda. A modified sedimentation technique was employed to detect the eggs of *Fasciola* species. In brief, faecal samples (app. 10 g) were crushed, diluted with 140 ml of saturated NaCl solution and filtered. The faecal suspension was transferred into a 15 ml test tube and sedimented for 20 minutes. Subsequently, the supernatant was discarded and the sediment was conveyed to a microscope slide using a pipette. To ease *Fasciola* spp. egg identification, a drop of Methylene blue was added and eggs were counted using a compound microscope with a 10× and 40× magnification (Hansen and Perry, 1994; Mwabonimana et al., 2009; Rojo-Vázquez et al., 2012). The identification of trematode eggs was facilitated by identification keys provided in Hansen and Perry (1994). *Fasciola* spp. prevalence was established as the number of infected individuals divided by the total number of samples taken in each herd (Margolis et al., 1982).

2.4 Image acquisition and processing

Four multispectral Sentinel-2 satellite images (European Space Agency, ESA) covering the entire Mutara rangelands (WGS 84, UTM zone 35S, EPSG code: 32735) during the infection season (i.e. the last wet season prior to faecal sampling) were downloaded from USGS Earth Explorer (https://earthexplorer.usgs.gov, last accessed on February 2019). Two images were taken during the short wet season (25 November 2015 and 24 January 2016), another two during the long wet season (14 March and 23 April 2016). All images had good quality, i.e. with little or no cloud cover, and were processed using QGIS (version 2.8.6). Prior to index calculation all sampling points were buffered using a radius of 1km as a proxy for the potential activity range of sampled herds (based on the average distance to the next water source; Apio, pers. comm.). Within these buffers, the NDVI, NDMI and NDWI (based on 10

× 10 m pixel size spatial resolution at earth surface) were established and averaged to obtain one value for each cattle herd.

2.5 Landscape metric variables

The rangeland proportion within each buffer area was determined using a high resolution Google satellite image. Along four radii (i.e. in direction to North, East, South, and West) in each buffer area, the distances intercepted by rangeland or agricultural fields were established. Subsequently, the rangeland proportion for each buffer was calculated. Building density was based on building registration data downloaded from 'Geofabrik' open street map (http://download.geofabrik.de, last accessed on February 2019). The number of buildings was established for each buffered area and divided by the total buffer size to obtain building density. Large scale rangeland grazing was reported to increase the *Fasciola* spp. infection risk by cattle being more exposed to vegetation contaminated by metacercaria (Kanyari et al., 2010; Murray and Daszak, 2013). By contrast, in more urbanized areas, livestock is mainly fed on freshly cut grass or agricultural waste and therefore exposed to a lower risk of *Fasciola* spp. infection (Kanyari et al., 2010).

2.6 Geological variables

Soil data were extracted from the pedological map of Rwanda (Van Ranst and Delvaux, 2000). The map was georeferenced and reclassified into two classes, i.e. poorly-drained soils *versus* well-drained soils. Buffered sampling areas were subtracted from the reclassified soil map and the proportion of poorly-drained soil was calculated. Elevation data were collected at each faecal sampling point using a hand-hold GPS. Alluvial soils and low elevations are usually associated to poor drainage and extended periods of flooding, prevalent in areas that correspond to increased *Fasciola* spp. prevalence in cattle (Zukowski et al., 1991; Malone and Yilma, 1999; McCann et al., 2010; Dutra et al., 2010; Bennema et al., 2011).

2.7 Animal husbandry variables

For each sampled cattle herd, the herd size and the adult proportion (i.e. the number of adults older than 24 months, divided by total herd size) were established. A visual BCS assessment based on estimating the presence or absence of musculature and fat deposition on the spinal and caudal vertebrae (El Alqamy, 2013) was applied to each sampled individual and subsequently a herd BCS was calculated by averaging scores. Livestock with deprived health condition or a poor nutritional status show usually a poor BCS, thus expecting a high *Fasciola* spp. prevalence.

2.8 Data analysis

Absolute data, were log-transformed, whereas relative data were arcsine square root transformed. To standardize data dimensionality, z-score normalisation was applied to the overall data set. The ten independent variables were reduced using Principal Component Analysis (PCA) resulting into four principle components with an Eigenvalue > 1.0, demonstrating 82.21% of the total variance. A univariate General Linear Model (GLM) was used to examine the impact of these risk factors on the *Fasciola* spp. prevalence in Ankole cattle by using the four PCs as covariates. Initially, all two-way interaction effects of all PCs, as well as a fixed factor (sampling season) and a random factor (herd ID) were included into the GLM, followed by a step wise backwards elimination procedure ($p > 0.1$) to omit non-significant interaction effects (all excluded interactions: $F < 1.50$, $p > 0.22$). Effect strengths were established as Wilk's partial eta-squared (ηp^2). All statistical analyses were carried out using *RStudio* (version 3.5.1)

3. Results

In total, 569 individuals from 142 cattle herds were sampled. Out of these, 113 individuals from 70 herds were detected positive for fasciolosis, corresponding to a total animal prevalence of 19.9% and a herd prevalence of 49.3%. Factor reduction using PCA of ten independent variables yielded four Principal Components (PCs, Table 1). PC1 obtained high factor loadings from NDVI, NDMI and NDWI, suggesting that areas covered by dense, woody vegetation corresponded to a high content of moisture in vegetation and soil and to only a few open water bodies. PC2 received high factor loadings from rangeland proportion, cattle herd size and building density, suggesting decreasing cattle herd size in areas where the original savannah vegetation was transformed into fields and human settlements. PC3 received high factor loadings from elevation and the proportion of poorly drained soil, indicating that poorly drained soils predominantly occur in areas of low elevation. PC4 obtained high factor loadings from the BCS and the proportion of adult individuals in the herd, suggesting that adult animals have generally a better body condition than juveniles.

A univariate GLM revealed that *Fasciola* spp. prevalence was significantly affected by several independent variables with a main positive effect of PC2, a main negative effect of PC4 (Table 2, Fig. 2a, b), and the interaction effect of 'PC1×PC3' and 'PC2×PC4' (Table 2, Fig. 3a, b). The *Fasciola* spp. prevalence showed no difference between the two sampling seasons (Table 2). Plotting the interaction effect 'PC1×PC3' generated two different slopes when dividing the data by the median of PC1. In the cohort of data with values loading on PC1 larger than the median (i.e., comparatively high NDVI, high NDMI, but low NDWI), we found *Fasciola* spp. prevalence to decrease with increasing values of PC3 (high elevation and well-drained soil, $R^2 = 0.023$; Fig. 3a). However, in the cohort of data with values of PC1 smaller than the median (i.e., comparatively low NDVI, low NDMI, but high NDWI) no such effect was found between *Fasciola* spp. prevalence and increasing PC3 ($R^2 < 0.001$; Fig. 3a).

Plotting the interaction effect ‘PC2×PC4’ also generated two different regressions when the data were separated by the median of PC2. In the cohort of data with values loading on PC2 larger than the median (i.e., comparatively high proportion of rangeland, large cattle herd size and low building density), *Fasciola* spp. prevalence slightly increased with increasing values of PC4 (adult proportion and averaged BCS, $R^2 = 0.006$; Fig. 3b), while in the cohort of data with values loading on PC2 smaller than the median (comparatively low proportion of rangeland, small cattle herd size and high building density), a strong effect was revealed between *Fasciola* spp. prevalence and the increasing PC4 ($R^2 = 0.249$; Fig. 3b).

4. Discussion

The overall *Fasciola* spp. prevalence observed in our study (49.3%) was relatively high compared to other studies on cattle in Rwanda (40.2%; Habarugira et al., 2016) or Ethiopia (32.3%; Bekele et al., 2010). However, depending on regional and seasonal factors the prevalence can vary considerably (Habarugira et al., 2016). Moreover, the *Fasciola* spp. prevalence was positively affected by PC2 (landscape metric variables and herd size, Table 2, Fig. 2a), indicating that large areas of original savannah vegetation, overgrazed by large cattle herds facilitated the spread of *Fasciola* spp.. Large stocking rates were previously reported to be the main reason for increased *Fasciola* spp. prevalence in cattle (Howell et al., 2015) and Morgan et al. (2006) suggested that low stocking rates are the prime measure to control the parasite in open grassland. Moreover, these grasslands are located in remote, rural areas with low building density, while in more urbanised areas with higher building densities and more agriculture, cattle herds are smaller and *Fasciola* spp. prevalence tends to be lower. The second main effect on *Fasciola* spp. prevalence was PC4 (adult proportion and BCS, Table 2, Fig. 2b), suggesting *Fasciola* spp. prevalence to decrease with increasing adult proportion and a high BCS. Moreover, the interaction effect of ‘PC2×PC4’ on the *Fasciola* spp. prevalence further highlighted how the landscape metric variables interacted with animal husbandry variables (Table 2, Fig. 3b). In urbanised, agricultural areas with a smaller proportion of rangeland and smaller cattle herds, but higher number of buildings (PC2 < median), the *Fasciola* spp. prevalence decreased with increasing adult proportion and higher BCS (Fig. 3b). However, there was no relationship between *Fasciola* spp. prevalence and PC4 (adult proportion and BCS) in rural areas with comparatively larger proportion of rangeland, larger cattle herds and lower building densities (PC2 > median; Fig. 3b). Here, cattle were heavily infected with *Fasciola* spp., regardless of age and body conditions. This result suggested that the animal husbandry variables (adult proportion and BCS) negatively correlated to *Fasciola* spp. prevalence only in urban areas, where cattle was fed on freshly cut grass or agricultural waste and thus interrupting the parasites’ life cycle. Land use changes in recent years led to increased urbanisation and the transformation of natural savannah vegetation into agricultural land (CIRAD, 2002; Wronski et al., 2017), reducing the availability of grassland for pastoralists and their cattle and thus reinforcing the negative effects of overstocking and overgrazing (Pandey et al., 1993; Taj et al., 2014).

Our GLM further revealed an interaction effect of ‘PC1×PC3’ on the *Fasciola* spp. prevalence (Table 2, Fig. 3a). Here, *Fasciola* spp. prevalence was not influenced by the geological variables if recorded in areas with comparatively less vegetation, less moisture but more open water bodies (PC1 < median, Fig. 3a). However, in dense woody vegetated areas with high moisture and few open water bodies (PC1 > median, Fig. 3a), high *Fasciola* spp. prevalence was correlated to poorly-drained soil and low elevation. This finding corresponds to our prediction that *Fasciola* spp. was prevalent in well-vegetated areas with high soil moisture and large proportions of poorly-drained soils at low elevations (Tum et al., 2004). Such specific environmental factors of the micro climate affect the presence and abundance of the intermediate host of *Fasciola* spp. (snails of the family Lymnaeidae) and thus determine the life-cycle of the parasite (Mzembe and Chaudhry, 1979; McCann et al., 2010; Charlier et al., 2014).

Given results from previous studies (Yilma and Malone, 1998; Malone et al., 1998; McCann et al., 2010; Kantzoura et al., 2011; Portugaliza et al., 2019), areas with sufficient vegetation (high NDVI), high moisture (high NDMI) or numerous open water bodies (high NDWI), i.e. areas facilitating the development of eggs, the mobility of miracidiae and the spread of cercariae, would be expected to show increased *Fasciola* spp. prevalence. Such areas include, flood plains and riverine forest, but also human-modified landscape elements like dams, swamps, ponds and irrigation canals. Dense vegetation with high soil moisture is known to be the ideal snail habitat (Tum et al., 2004; Malone, 2005), and remote sensing indices, such as NDVI and NDMI, were frequently used to assess the environmental variables typical for snail habitats, to identify high risk *Fasciola* spp. areas and to develop regional fasciolosis risk maps (Malone et al., 1998; Durr et al., 2005). However, the hypothesis that high *Fasciola* spp. prevalence occurs in areas with a high density of open water bodies, i.e. a high NDWI, was not proven by our study.

5. Conclusions

The prevalence of *Fasciola* spp. in Ankole cattle was, at least to a certain degree, defined by all independent variables included in our study. In contrast to other gastro-intestinal parasites (e.g. *Eimeria* spp. or strongyle-type nematodes), the intermediate host and the free-living stages of *Fasciola* spp. require habitats covered by dense and lush vegetation with large proportions of poorly-drained soils at low elevations. Therefore, such habitats should be considered as high fasciolosis risk areas for grazing cattle. Our results further confirmed that the land use changes of the Mutara rangelands in recent decades, i.e. increased urbanization and subsistence agriculture, correspond to a reduced availability of space for the pastoralist community, leading to increased overstocking and overgrazing and thus making the Mutara rangelands an unbalanced and unhealthy ecosystem (e.g. increased fasciolosis). In the future, more random sampling across the Mutara rangelands (or the entire country) is needed to prepare a predictive, spatial fasciolosis risk map, which would help to monitor *Fasciola* spp. dispersal routes, and to develop sustainable land-use management strategies that improve the health of humans, their livestock and the ecosystem in which they live.

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Figure legends

Fig. 1 Location of the study area (three 2.5 km wide transect belts) in Nyagatare District in northern Rwanda. Each sampling location (dots) was aligned to a buffer area of 1km radius (upper right inset) for which independent variables were determined.

Fig. 2 The relationships of PC2 (a) and PC4 (b) with the *Fasciola* spp. prevalence in Ankole cattle on the Mutara rangelands.

Fig. 3 a. Scatter plot showing the interaction effect of “PC1×PC3” on *Fasciola* spp. prevalence: No relation was unrevealed between *Fasciola* spp. prevalence and increasing PC3 as seen in case of the data with values loading on PC1 smaller than the median (shaded dots, grey dashed line; linear regression: $R^2 < 0.001$), while decreasing *Fasciola* spp. prevalence with increasing values of PC3 become evident for the data with values loading on PC1 larger than the median (bold dots, black line; linear regression: $R^2 = 0.023$).

b. Scatter plot showing the interaction effect of “PC2×PC4” on *Fasciola* spp. prevalence: Distinctly decreasing *Fasciola* spp. prevalence with increasing PC4 is seen in case of the data with values loading on PC2 smaller than the median (shaded dots, grey dashed line; linear regression: $R^2 = 0.249$), while slightly increasing *Fasciola* spp. prevalence with increasing values of PC4 become evident for the data with values loading on PC2 larger than the median (bold dots, black line; linear regression: $R^2 = 0.006$).

Tables

Table 1 Axis loadings of four principal components (demonstrating 82.21 % of the total variance), obtained from principal component analysis of ten independent variables (see section 3.2). PC loadings > |0.5| are shown in bold font type.

Principal component	**PC1**	**PC2**	**PC3**	**PC4**
Eigenvalue	3.21	2.20	1.46	1.36
Percent variance	26.290	25.62	16.15	14.15
NDVI	**0.953**	0.219	-0.066	-0.032
NDMI	**0.894**	-0.163	-0.240	0.054
NDWI	**-0.892**	-0.275	-0.092	0.039
Rangeland proportion	0.170	**0.934**	0.060	-0.031
Herd size	-0.042	**0.872**	0.124	-0.100
Building density	-0.187	**-0.863**	0.229	0.012
Elevation	0.064	-0.117	**0.873**	-0.041
Poorly drained soil proportion	0.230	-0.117	**-0.840**	-0.022
BCS	-0.047	-0.019	0.039	**0.839**
Adult proportion	0.036	-0.083	-0.060	**0.832**

Table 2 Results of the univariate GLM using the *Fasciola* spp. prevalence as the dependent variable, sampling season as a fixed factor and the four principal components (PCs) as covariates. Insignificant interaction effects were excluded if $p > 0.1$.

variables	**Estimate**	**SE**	***t***	***p***	**Partial eta^2**
Sampling season	-0.286	0.170	-1.680	0.095	0.021
PC1	-0.058	0.085	-0.687	0.493	0.008
PC2	0.248	0.083	2.967	**0.004**	0.080
PC3	-0.065	0.076	-0.850	0.397	0.005
PC4	-0.215	0.081	-2.654	**0.009**	0.095
PC1×PC3	-0.184	0.083	-2.232	**0.027**	0.036
PC2×PC4	0.245	0.072	3.383	**<0.001**	0.079