Objectifs du travail de la thèse

3.1. Objectif général

La fièvre aphteuse est enzootique dans presque tous les pays d'Afrique Subsaharienne dont le Tchad (Couacy-Hymann et al. 2006; Gueme et Bidjeh 1998; Paton, Sumption, et Charleston 2009; Ouagal et al. 2010; Rweyemamu et al. 2008; Bachir et al. 2018). Mais malgré l'endémicité, la capacité du virus à se propager rapidement et l'impact négatif que la fièvre aphteuse pourrait avoir sur le développement de l'élevage, au Tchad, elle n'a pas été considérée comme une maladie prioritaire. Pendant plusieurs années, la maladie a été sous-déclarée et incontrôlée au Tchad, comme dans des nombreux pays de l'Afrique centrale.

Comme maintenant, il y a de plus en plus d'exigence dans la qualité de produits d'origine animale lors de la demande sous-régionale, y compris le bétail vivant, donc pour exporter, il faut se conformer aux normes internationales. La réponse à ces exigences est incontestablement l'amélioration de l'état sanitaire du bétail par le renforcement du système national de surveillance épidémiologique des maladies animales et de contrôler les maladies transfrontières, y compris la fièvre aphteuse (Souley Kouato 2017). Cependant, il existe des preuves que la fièvre aphteuse est présente au Tchad mais elle est peu étudiée, car il a été trouvé quelques données sur la séroprévalence de cette maladie dans la littérature (Gueme et Bidjeh 1998; Ouagal et al. 2017; Ouagal et al. 2018). Ainsi, une meilleure connaissance de l'épidémiologie moléculaire de la fièvre aphteuse est déterminante pour la mise en œuvre de mesures de lutte efficaces.

L'objectif général de cette thèse de doctorat est de **contribuer à l'amélioration des connaissances de l'épidémiologie de la fièvre aphteuse au Tchad pour mettre en œuvre un programme national de lutte contre cette maladie**.

3.2. Objectifs spécifiques

Les objectifs spécifiques sont :

- évaluer et cartographier les zones à risque d'occurrence de la fièvre aphteuse en lien avec la mobilité animale ;
- déterminer la séroprévalence et effectuer la caractérisation moléculaire du virus de la fièvre aphteuse ;

- proposer des recommandations pour la lutte contre la fièvre aphteuse et des perspectives pour des études ultérieures dans le pays à prendre en considération.

Tout ceci, implique d'une part, la compréhension de l'analyse qualitative du risque et de la nouvelle approche cartographique du risque en lien avec la mobilité animale ; et d'autre part, de la connaissance des tests sérologiques (tests ELISA FMDV) et des tests virologiques tels que la RT-PCR, le séquençage nucléotidique du virus suivi d'une analyse phylogénétique afin de comprendre l'épidémiologie moléculaire de la fièvre aphteuse au Tchad.

Chapitre 4 : Séroprévalence et caractérisation moléculaire du FMDV au Tchad

Résumé :

Cette étude visait à déterminer la séroprévalence de la fièvre aphteuse chez les ruminants domestiques et à caractériser les souches virales en circulation dans trois régions du Tchad (Batha, Wadi Fira et Ennedi Ouest). Au total, 1520 échantillons de sérum (928 de bovins, 216 de caprins, 254 d'ovins et 122 de dromadaires) ont été prélevés et testés par le test ELISA PrioCHECK® FMDV NSP, pour détecter la présence d'anticorps induits par des protéines non structurales du virus de la fièvre aphteuse (en particulier la protéine 3ABC), correspondant à une infection passée par le virus de type sauvage. 9 échantillons de tissus épithéliaux des bovins ont été également prélevés pour l'isolement et la caractérisation du virus de la fièvre aphteuse. Les résultats sérologiques du test ELISA PrioCHECK® FMDV NSP montrent une séroprévalence globale de 40 % (602/1520), IC à 95 % [19-63]. Cependant, les séroprévalences de 84 %, 78 % et 84 % ont été estimées chez les bovins de plus de 5 ans respectivement aux Batha Est, Batha Ouest et Wadi Fira. Chez les bovins de moins d'un an, la séroprévalence était estimée à 67 % au Wadi Fira, 64 % au Batha Est et 59 % au Batha Ouest. Il a été constaté que ces taux de séroprévalence élevés obtenus dans les différentes zones étaient liés à des facteurs de risque favorisant la propagation de la maladie tels que le partage des pâturages avec d'autres troupeaux voisins, la combinaison de l'élevage bovin et ovin et l'âge des animaux. Les résultats du test de sérotypage ELISA PrioCHECK® FMDV types O et A et du test ELISA de compétition en phase solide (SPCE) (pour détecter les types SAT 1 et SAT 2) réalisés sur les sérums des jeunes animaux âgés de moins d'un an qui sont positifs au test ELISA PrioCHECK® FMDV NSP, montrent que les quatre sérotypes O, A, SAT 1 et SAT 2 ont circulé au Tchad en 2015. Cependant, le type SAT 2 dominait avec une séroprévalence globale de 43,3 % et était présent dans toutes les régions étudiées. Les analyses virologiques ont permis de caractériser le sérotype SAT 2 de la fièvre aphteuse et les analyses phylogénétiques de la séquence codante VP1 ont permis de déterminer le sérotype SAT 2 topotype VII, proche des souches virales trouvées au Cameroun en 2015 avec une similarité de 98,60%.

Seroprevalence and molecular characterization of Foot-and-Mouth-Disease virus in Chad

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Abstract:

This study aimed to determine the seroprevalence of Foot-and-Mouth Disease (FMD) in domestic ruminants and to characterize the virus strains circulating in three regions of Chad (Batha, Wadi Fira and West Ennedi). In total, 1520 sera samples (928 from cattle, 216 from goats, 254 in sheep and 122 from dromedaries) were collected for FMD serological analyses. Nine samples of bovine epithelial tissue were also collected for the isolation and characterisation of the foot-and-mouth disease virus. Serological results show an overall seroprevalence of 40%, 95% CI [19-63]. However, the seroprevalences of 84%, 78% and 84% were estimated in cattle over 5 years of age in East Batha, West Batha and Wadi Fira respectively. In cattle under one year of age, 67% seroprevalence was estimated in Wadi Fira, 64% in East Batha and 59% in West Batha. It was found that these high seroprevalence rates obtained in the different areas were linked to risk factors favouring the spread of the disease such as grazing sharing with other neighbouring herds; the combination of cattle and sheep breeding and the age of the animals. ELISA PrioCHECK® FMDV types O and A and in-house solid phase competition ELISA (to detect types SAT 1 and SAT 2) serotyping results show that the four O, A, SAT 1 and SAT 2 serotypes circulated in Chad in 2015. However, the type SAT 2 dominated with an overall seroprevalence of 43.3%, and was present in the all regions investigated. The virological analyses permitted characterization of FMD serotype SAT 2. The phylogenetic analyses of the VP1 coding sequence allowed determining the serotype SAT 2 topotype VII, close to viral strains found in Cameroon in 2015 with a similarity of 98.60%.

Keywords: Foot-and-mouth-disease virus; Chad; molecular characterization; seroprevalence

Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease caused by a virus of the genus *Aphthovirus* within the *Picornaviridae* family. It especially affects wild and domestic *Artiodactyles*, namely cattle, sheep, goats and pigs (Jamal & Belsham, 2013). FMD virus (FMDV) is a small virus consisting of a single-stranded RNA genome of approximately 8500 bases encoding for structural and non-structural proteins. The virion is icosahedral, non-enveloped with a positive-sense single-stranded RNA genome (Domingo et al., 1990; Thiry, Baranowski, & Domingo, 2001). There are seven distinct serotypes (O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1). Each serotype has multiple subtypes, because of the great antigenic variability resulting from genetic variation during FMDV replication (OIE, 2017).

FMD is enzootic in most of Africa, particularly in Central and West Africa (Bachir et al. 2018; Couacy-Hymann et al. 2006; Paton, Sumption, et Charleston 2009; Rweyemamu et al. 2008; Souley Kouato et al. 2018). Its occurrence is mainly associated with the presence of domestic ruminants and animal mobility (Bouslikhane 2015). In Chad, four serotypes have been identified: O, A, SAT 1 and SAT 2 (Gueme et Bidjeh 1998; Ouagal et al. 2010; Ouagal et al. 2017). This serotypes also circulate in neighbouring countries: Sudan, Niger, Libya and Cameroon (Abu Elzein 1983; Abubakar et al. 2017; Bachir et al. 2018; Bertram et al. 2018; Ehizibolo et al. 2017; Eldaghayes et al. 2017; Habiela et al. 2010; Kouato et al. 2018).

In Chad, despite the endemicity of the disease, few studies have been carried out on seroprevalence (Ouagal et al., 2018; Ouagal et al., 2017) and no studies on the molecular characterisation of the FMDV have been carried out before. That is why we have decided to carry out a study within the framework of the Pastoral Livestock Reinforcement Project in Chad (PREPAS) to determine not only the seroprevalence of FMD but also to characterize the serotypes of the FMDV circulating in the area covered by this project.

Matreial and Methods

Study area and sampling

The study area includes three livestock regions of Chad: Batha, Wadi Fira and West Ennedi (Figure 10). These areas are known fpr having favourable climatic conditions during the rainy season (July-October) for animal grazing, and therefore gathers most nomadic herds (cattle, small ruminants and dromedaries) from the subregion.

A cross-sectional survey for sample collection was conducted in October and November 2016. Chad's territorial administration is divided into regions that are divided into departments, while the latter are divided into sub-prefectures. These sub-prefectures are divided into cantons. Thus, the epidemiological unit selected for carrying out the surveys was the canton. On the basis of animal density, twenty cantons were sampled: six cantons in East Batha, six cantons in West Batha, four cantons in Wadi Fira and four cantons in West Ennedi. In East Batha and West Batha, eight cattle herds, three goat and sheep herds and one dromedary herd were sampled per canton. In Wadi Fira, five cattle herds, and three goat, sheep and camels herds were sampled. Finally, four goat and sheep herds, and five dromedary herds were sampled in West Ennedi.

A minimum of 11 sera per herd of cattle and 5 sera per herd of small ruminants and dromedaries were sampled to reach 95% chance to observe at least one positive test in the herd, assuming random sampling within herds with seroprevalence rates of 25% and 50% respectively in cattle and small ruminants. In total, 1520 sera samples were collected for serological analyses (928 from cattle, 216 from goats, 254 from sheep and 122 from dromedaries). Nine samples of bovine epithelial tissue were also collected for the isolation and characterisation of the foot-and-mouth disease virus. Samples were sent according to the recommendations of the OIE (OIE, 2008) to the FMD reference laboratory of Maisons-Alfort (France), for confirmatory diagnosis and FMDV serotype characterization.

Laboratory analyses

Three serological tests (ELISA PrioCHECK® FMDV NSP, ELISA PrioCHECK® FMDV types O and A and ELISA internal solid phase competition (SPCE)) were performed in this study. The first test was the PrioCHECK® FMDV NSP ELISA was used to determine the overall seroprevalence of FMD. This test is not specific to serotypes. It detect the presence of antibodies induced by non-structural proteins (in particular 3ABC protein) (Brocchi et al., 2006; Sørensen et al., 1998). The second test was the ELISA PrioCHECK® FMDV types O and A was used to detect antibodies against FMDV O and A serotypes (Hamblin, Barnett, & Hedger, 1986; Relmy et al., 2017; Van Maanen & Terpstra, 1989) for FMDV serotyping. The third test was the solid phase competition ELISA (SPCE) test was used for the detection of antibodies against FMDV serotypes SAT 1, SAT 2 and SAT 3 (Li et al. 2012). The reference strains of FMDV SAT 1 ZIM 25/89, SAT 2 ZIM 3/97, SAT 3 ZIM 4/99 were used as antigens in this SPCE test (Relmy et al., 2017). The sensitivity (Se) and specificity (Sp) of these

PrioCHECK® commercial tests have already been known. They are given by the supplier : ELISA PrioCHECK® FMDV NSP (Se :96% et Sp :87%), ELISA PrioCHECK® FMDV type O (Se :95% and Sp :99,4%), ELISA PrioCHECK® FMDV type A (Se :90% and Sp :99%). For the ELISA SPCE, the Se is 100% and the Sp is 99.41-99.7% (Li et al. 2012). For FMDV detection, three following tests were performed. The first test was the Real-time RT-PCR for FMD detection targeting the 3D and IRES regions of the viral genome (Laor, Torgersen, Yadin, & Becker, 1992; Reid, Ferris, Hutchings, Samuel, & Knowles, 2000). The second test was the RT-PCR multiplex was used for FMDV typing (Callens & De Clercq, 1997; Giridharan, Hemadri, Tosh, Sanyal, & Bandyopadhyay, 2005; Gorna et al., 2016). However, this test does not allow the sequencing, because the amplified part is very short (250 nt) which is insufficient for complete sequencing of the VP1 gene (Gorna et al., 2016). The third test was the Conventional RT-PCR has been carried out for amplification of the complete gene encoding the VP1 protein (Relmy et al. 2017). Indeed, specifics primers of FMDV serotype SAT2 (P1-1223F / SAT-2B208R) were chosen to amplify this region of the genome encoding the VP1 protein (Ayelet et al., 2009).

Bioinformatic analysis of nucleotide sequences

The sequences obtained were assembled and verified using ContigExpress software (Vector NTI, Invitrogen). A complete sequence of the VP1 coding region was analysed and compared to the homologous genomic regions available in the NCBI GenBank database. Multiple sequence alignment and phylogenetic analyses were conducted using MEGA version 6 software (Tamura et al. 2013). FMDV sequences were aligned using the Muscle program with default parameters (Edgar, 2004). Phylogenetic analyses were conducted by Neighbor-Joining (NJ) method (Saitou & Nei, 1987) with the Kimura 2-parameter model (Kimura, 1980). The confidence of the NJ tree was assessed by bootstrap analysis with 1000 replicates.

Statistical analyses

Binomial regression was conducted using the package R-INLA (Rue, Martino, & Chopin, 2009) of the R software (R Core Team, 2018) to estimate overall disease seroprevalence by species, age category and region. Since infection patterns are very different between species, species-specific seroprevalence were estimated. However, one estimate was presented for small ruminants that is sheep and goats.

The number of positive groups y_{ij} in region i and age-category j for a given species has been modelled with a binomial likelihood $y_{ij} \sim Bi(n_{ij}, p_{ij})$

where n_{ij} is the number of sampled groups in region i and age-group j.

The group-prevalence p_{ij} has been modelled in a logit-scale with a linear predictor with a region-specific intercept β_i and varying effects w_{ij} of the age-category for each region:

$$y_{ij} = \log(\frac{p_{ij}}{1 - p_{ij}}) = n_{ij} = \beta_{ij} + w_{ij}$$

$$w_{\cdot j} \sim N(0, AR1(\sigma_w, \varphi))$$

so that within each region, a separate first-order autoregressive model (AR1) has been used for the effect of the age-category, all sharing the same marginal variance σ_w and autocorrelation parameter φ

Given the small number of groups for some species (e.g. less than 10 for most age-categories and regions of small ruminants), we fitted this model using Bayesian approach in order to reduce overfitting and improve the seroprevalence estimates. Finally, for the prior parameter of the scale, the approach of the penalized complexity prior was followed. This approach is defined in terms of the Kullback-Leibler distance from the model from a simpler model with zero variance. The penalty rate is determined by specifying a probability assessment such that P(σw > U) = α (Simpson, Rue, Riebler, Martins, & Sørbye, 2017). Thus, to make the calculation, U = 3 and α = 0.001 were set, which means that it is very unlikely that the scale parameter of the autoregressive effect was greater than 3.

Results and Discussion

Seroprevalence results

Figure 11 shows observed (black) and estimated (grey) seroprevalences at the group level by region and age category for the different animal species studied. Serological results show an overall seroprevalence of 40%, 95% CI [19-63].

Indeed, in cattle over 5 years of age, seroprevalence was estimated at 84% (95% CI: 72 - 93), 78% (95% CI: 65 - 89), 84% (95% CI: 69 - 95) respectively in East Batha, West Batha and Wadi Fira. Aslo, seroprevalence estimates were made for cattle under one year of age: 67%

(95% CI: 48 - 83) in Wadi Fira, 64% (95% CI: 45 - 81) in East Batha and 59% (95% CI: 41 - 76) in West Batha.

Furthermore, among small ruminants over 5 years of age, seroprevalences of 63% (95% CI: 36 - 88), 45% (95% CI: 21-70), 34% (95% CI: 16-53) and 10% (95% CI: 2-23) have been estimated in West Batha, East Batha, Wadi Fira and West Ennedi respectively. Also, among young small ruminants under one year of age, seroprevalences was estimated in the different areas studied: 32% (95% CI: 12-55) in East Batha, 18% (95% CI: 4-39) in West Batha, 50% (95% CI: 25-76) in Wadi Fira and 11% (95% CI: 3-27) in West Ennedi.

Finally, seroprevalence has also been estimated among dromedaries over 5 years old in the different areas: 33% (95% CI: 10-56) in East Batha, 20% (95% CI: 4-38) in West Batha, 16% (95% CI: 5-36) in Wadi Fira, 7% (95% CI: 2-16) in West Ennedi. In dromedaries less than 1 year old, seroprevalence was not determined due to lack of samples in this age group.

Based on the results of the seroprevalences estimates obtained, it was found that the three areas: West Batha, East Batha and Wadi Fira have high seroprevalence of FMD compared to the West Ennedi area. This can be explained by the fact that these three areas have high animal densities (cattle and small ruminants). In addition, these are transhumant areas where animal movements are uncontrolled. These uncontrolled movements may be one of the main sources of disease spread. Our results are consistent with previous results obtained by (Ouagal et al. 2010) in the same areas in Chad. On the other hand, the low seroprevalence rate obtained in Ennedi West could be explained by the fact that this area is arid, with a very low animal density and limited between herds contacts.

The low seroprevalence of dromedaries compared to other animal species (cattle, goats and sheep) is due to the fact that camels are less susceptible to FMD. Our results on the seroprevalence of FMD in dromedaries confirm the results obtained by (Wungak et al., 2015) in Nigeria.

Seroprevalence rates by age group were found to be higher in older animals than in young animals under one year of age. These low seroprevalence rates in young animals (less than one year old) may be due to low exposure of young animals to risk factors or to the fact that the virus has recently circulated at a low level. There is also the practice of herders to keep young animals separate from adult animals around the village, which may also explain the

low seroprevalence rates. This may also be due to the fact that adult animals are repeatedly exposed and in close contact with other animals in pastures and water points. Our results are similar to those of (Wungak, Olugasa, Ishola, Lazarus, & Ularamu, 2016). These authors determined seroprevalence by age group in Nigeria and also obtained higher seroprevalence in adults than in young animals.

Concerning the serotyping of the FMDV in Chad, serotyping tests were carried out on sera from young animals under one year of age (67/194) positive for the NSP ELISA test. The FMDV serotyping results have been broken down by geographical area and are presented in Table I. These results show that the four serotypes O, A, SAT1 and SAT2 circulated well in Chad in 2016. The SAT 2 type dominated with an overall seroprevalence of 43.3% and was present in all four areas. It is followed by serotype O (29.9%). This serotype O was present in West Batha and Wadi Fira. Then comes serotype A (22.4%) but it has only been detected in Wadi Fira. Finally, the SAT 1 type (4.5%), it was present in East Batha and West Batha.

The presence of the four serotypes (A, O, SAT 1 and SAT 2) in the country could be due to the free cross-border movement of animals in search of pastures and water points and uncontrolled trade in livestock between Chad, the Central African Republic, Sudan, Cameroon and Niger. Indeed, these four circulating serotypes (O, A, SAT 1 and SAT 2) have also been identified and not yet eradicated in countries bordering Chad (Bachir et al., 2018; Bertram et al., 2018; Habiela et al., 2010; Kouato et al., 2018; Wungak et al., 2016).

Virological results

Virological analyses initially yielded 5/9 positive samples to the real-time RT-PCR test detecting both IRES and 3D targets of the viral genome. Then, the five real-time RT-PCR positive samples were by tested conventional multiplex RT-PCR for virus typing. These typing results showed that only one in five was positive, confirming the presence of FMDV serotype SAT 2. This serotype SAT 2 sample was taken from the West Batha area. Several attempts to isolate and amplify the VP1 gene were made but without success on the four negative samples. This may be explained by the poor quality of these samples.

Finally, phylogenetic analyses of the sequence coding for VP1 of the SAT 2 type obtained made it possible to determine the SAT 2 topotype VII serotype, close to the virus strains of Cameroon 2015 with a similarity of 98.60% (Figure 12). This suggests the movements of infected animals between Chad and Cameroon.