# Seroprevalence and risk factors for peste des petits ruminants in sheep and goats in Djibouti

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**Summary**

Despite the occurrence of peste des petits ruminants (PPR) in all other countries in the Horn of Africa, which engage in free animal movement, to date, PPR has not been reported in Djibouti. The objective of this study was to estimate the seroprevalence of PPR and its associated risk factors in sheep and goats in that country. A cross-sectional method was used with proportional sampling to allocate the number of small ruminants to be sampled from each of the country's regions (Ali Sabieh, Arta, Dikhil, Djibouti, Obock and Tadjourah). From a total of 1,516 serum samples tested using a competitive enzyme-linked immunosorbent assay (cELISA), 91 were positive, with an overall 6% (95% confidence interval [CI] = 4.8–7.2) prevalence of antibodies to the peste des petits ruminants virus (PPRV). Antibodies to PPRV were detected in small ruminants from all the regions, excluding Obock. Seroprevalence was highest in the Tadjourah region (8.92%), whereas the lowest prevalence was observed in the Djibouti region (1.28%). The species, age and sex of the animals, and the herd size were identified as risk factors for PPR

seropositivity. The risk of goats testing positive for PPRV antibodies was 2.95 (CI = 1.39–6.35) times that of sheep. Moreover, the risk of animals younger than two years of age testing positive for PPRV antibodies was 2.29 (CI = 1.47–3.56) times that of animals older than two years of age. Similarly, it was shown that female animals were more frequently infected (odds ratio [OR] = 3.82; CI = 1.51 to 9.67) than their male counterparts. In addition, small ruminants from small-sized herds/flocks were more likely to be seropositive (OR = 2.06; CI = 1.10–3.83) than those from medium-sized herds/flocks. The present study revealed, for the first time, the widespread occurrence of PPRV antibodies in small ruminants in Djibouti with low prevalence.

**Keywords**

Competitive enzyme-linked immunosorbent assay – cELISA – Djibouti – Goats – Peste des petits ruminants – PPR – Seroprevalence – Sheep.

## Introduction

Peste des petits ruminants (PPR) is an acute viral disease of small ruminants characterised by fever, oculo-nasal discharges, stomatitis, diarrhoea and pneumonia with high morbidity and mortality (1). It is caused by the peste des petits ruminants virus (PPRV) of the genus *Morbillivirus* belonging to the family *Paramyxoviridae* (2, 3). Among domestic animals, clinical PPR is most common in sheep and goats, with goats appearing to be more susceptible than sheep. It has also been shown that differences in susceptibility exist between different breeds and ages of goats (4). Although less frequently, clinical PPR does occur in camels under natural infection (5). Wild small ruminants are also susceptible to PPR, and the disease results in a huge burden due to the weight loss and death it causes in affected animals (6, 7). Cattle, however, are generally affected subclinically, although fatal cases have been observed in India in experimentally infected calves (6).

The disease was first described in Côte d'Ivoire in 1942, and not long thereafter it had been reported in several sub-Saharan African

countries (4). Its distribution in Africa extends as far south as Angola and the Democratic Republic of the Congo, and as far north as Morocco, Tunisia and Algeria. The disease has also been confirmed in Eastern Africa and all Inter-Governmental Authority on Development (IGAD) Member States except Djibouti (i.e. Eritrea, Ethiopia, Kenya, Somalia, the Republic of South Sudan, Sudan and Uganda) (8). In addition, PPR was confirmed in Comoros in January 2012. Moreover, its occurrence outside Africa has been confirmed in the Middle East and in several Asian countries (4). Currently, 70 countries have confirmed the occurrence of the disease in their small ruminant populations, the majority of these countries are in Africa, where 50 other countries are at risk of acquiring the disease (9).

In Djibouti, small ruminants account for more than 80% of the livestock population, and play significant economic and social roles. These animals are considered key resources through which to achieve food security, and, together with other livestock species, they provide employment opportunities for over 150,000 people (10). Even though Djibouti is the only IGAD Member State which has not yet reported a case of PPR, the disease has been reported in all neighbouring countries. Djibouti shares borders with Eritrea, Ethiopia and Somalia, and cross-border livestock movement in search of pasture and water is common in the region. Therefore, there is potential for the disease to be introduced into the country. During the course of this study, clinical cases of PPR have not been reported. However, to benefit from the global PPR control and eradication programme launched in 2014, which covers all IGAD Member States, documented proof of the disease in the country is needed. This study was, therefore, carried out with the objective of assessing the epidemiology of PPR in sheep and goats in Djibouti using a competitive enzyme-linked immunosorbent assay (cELISA).

## Materials and methods

### Study area

The study was conducted in the six administrative regions of Djibouti, namely Ali Sabieh, Arta, Dikhil, Djibouti, Obock and Tadjourah

(Fig. 1). The country is bordered by Ethiopia to the west and south, Eritrea to the north, and Somalia to the south-east. The remainder of the border to the east is made up of the Red Sea and the Gulf of Aden. Geographically, Djibouti is located between 10° and 13°N latitude, and 41° and 44°E longitude. Its total land area is 23,200 $km^2$ supporting over 1,024,194 inhabitants. Djibouti experiences dry weather ranging from arid in the north-eastern coastal regions to semi-arid in the central, northern, western and southern regions. The annual rainfall of Djibouti ranges from 131 mm on the eastern seaboard to 300 mm in the central regions. The rainfall pattern is seasonal with the wet season extending from October to April and the dry season from May to September. The annual average maximum and minimum temperature range from 39°C to 42°C and 21°C to 25°C, respectively (10, 11).

## Study design

A cross-sectional study was carried out between November 2015 and July 2016 on sheep and goats reared in the six regions of Djibouti. Proportional sampling was used in this study. Three or more villages were selected from each of the six administrative regions. Small ruminants found in the selected villages were sampled using random sampling techniques. In addition to serum sample collection, a questionnaire was used to collect information on the herd owners' awareness of the disease and the risk factors for PPR occurrence in the areas studied. The information collected includes the practice of using communal grazing and watering points, the movement of animals to market and for congregation at night. Flock owners were interviewed to obtain information on herd size, the introduction of new animals, the production system, grazing management, housing, water sources and access to veterinary services. During serum sample collection, the age of each animal was estimated using dentition, and the animals were classified as young (six months to ≤ 2 years) and adults (> 2 years). Herd size was also classified as small sized (< 100 animals), medium sized (100–200 animals) and large sized (≥ 200 animals).

## Study population and sample size determination

The study population comprises small ruminants raised in the six administrative regions of Djibouti. Since the occurrence of PPR has not been reported in the country, the animals included in this study were not vaccinated against the disease. All of the study animals were over six months of age. Serum samples were collected from a total of 1,516 sheep and goats. Proportional sampling was used to allocate the number of small ruminants to be sampled from each region. As no previous study had been undertaken in Djibouti, the overall sample size needed was determined using a formula described by Thrusfield (12), using the parameters of 5% absolute precision, 95% confidence interval level and 50% expected prevalence. However, since cluster sampling was used in each village, the sample size increased nearly fourfold.

## Blood sample collection and transportation

A total of 1,516 serum samples were collected from 301 sheep and 1,215 goats. Blood samples of approximately 5 ml were collected from the jugular vein using plain vacutainer tubes and sterile 18 gauge needles. Each sample was labelled separately indicating the animal species and sampling area. The samples were placed in a slant position at room temperature to allow for the clotting of blood and ease of separation of serum. After retraction of the clots (60 min), each sample was centrifuged and the clear serum was collected into 4 ml cryovials and transported to the National Laboratory of Animal Disease Diagnostics of Djibouti in an ice box containing ice packs. The samples were then stored at −20°C until processed.

## Laboratory analysis by a competitive enzyme-linked immunosorbent assay

A monoclonal antibody based cELISA described by the World Organisation for Animal Health (OIE) and Diallo *et al.* (6, 13), was used for the detection of antibodies directed against the nucleoprotein (NP) of the PPRV using a standard kit (14), following the manufacturer's instructions (French Agricultural Research Centre for

International Development [CIRAD], Paris, France). In brief, the wells of the enzyme-linked immunosorbent assay (ELISA) microplates were coated with purified recombinant PPR NP, and the samples to be tested and the controls were added to the wells. Next, an anti-NP antibody–horseradish peroxidase (HRP) conjugate was added to the microwells and the microplates were incubated. After washing, according to the ID.Vet protocol (ID.Vet, Grabels, France) the chromogenic substrate solution (3,3',5,5'-Tetramethylbenzidine or TMB) was added and the resulting colouration was dependent on the quantity of specific antibodies present in the sample. It was incubated for 15 min ± 2 at 21°C (±5°C) in the dark and then a stop solution was added to each well in order to terminate the reaction. The microplates were read at a wavelength of 450 nm using a BioTek® ELISA plate reader that was connected to a computer running BioTek® Gen 5™ software for automated reading (BioTekInstruments, Inc., Winooski, Vermont, United States of America). For each sample, the optical density (OD) was calculated as competition percentage values. Interpretation of the OD values was made following the manufacturer's instructions. Positive and negative controls provided with the kit were used to validate the test procedure.

### Data management and analysis

Laboratory results and field information collected during the sampling were analysed using STATA version 13 (StataCorp, College Station, Texas, United States of America). Descriptive statistical analysis, such as the Chi-squared test (Fisher's exact test) was used to quantify the association between the seroprevalence and categorical risk factors. Multivariable logistic regression was used to analyse the effect of multiple risk factors on the seroprevalence of PPR. A 95% confidence level and a 5% significance level were considered appropriate for the interpretation of statistical analysis.

## Results

From a total of 1,516 serum samples tested using cELISA, 91 were found positive, yielding an overall prevalence of anti-PPRV antibodies of 6% (95% confidence interval [CI] = 4.8–7.2) in small

ruminants in Djibouti. The true prevalence of anti-PPRV antibody was 5.75%. Table I presents the results of seroprevalence in Djibouti. Antibodies to PPRV were detected in small ruminants from all regions sampled, except region Obock. The seroprevalence was highest in the Tadjourah region (8.92%), whereas it was lowest in the Djibouti region (1.28%). Seroprevalence in goats (6.83%) was higher than that in sheep (2.66%). Similarly, higher seroprevalence was observed in female animals (6.68%) than in male animals (2.18%). Univariable analysis showed that factors such as region; the species, sex and age of the animals; the production system, grazing and water sources were associated with the seroprevalence of PPR in small ruminants in Djibouti.

The results of multivariable logistic regression analysis showed that only the species, sex and age of the animals, and herd size are risk factors for the seroprevalence of PPR in Djibouti (Table II). Moreover, goats were nearly three times more likely to be PPR seropositive than sheep (OR = 2.95; CI = 1.39–6.35). Animals younger than two years of age were found to be 2.29 times more likely to be PPR seropositive than animals that were older than two years (OR = 2.29; CI = 1.47–3.56). Furthermore, female animals are more frequently infected than their male counterparts. That is, the odds of being infected with PPRV was nearly four times higher in females than in males (OR = 3.82; CI = 1.51 to 9.67). In addition, small ruminants from medium-sized herds/flocks were 2.06 times more likely to be seropositive than those from small-sized herds/flocks (OR = 2.06; CI = 1.10–3.83). Finally, no statistically significant difference in risk of infection was revealed for husbandry system, grazing and water sources.

## Discussion

Peste des petits ruminants, an acute contagious disease affecting mainly small ruminants is considered one of the priority diseases in Djibouti. The present study provides the first report of anti-PPRV antibody in small ruminants in the country with an overall apparent prevalence of 6% (true prevalence of 5.75%). Seropositivity was

detected in all but one region. Therefore, this shows that infection with PPRV is widespread within Djibouti. However, the seroprevalence observed in this study is low, but potential risk factors for PPR transmission may remain as a constant threat to small ruminants in Djibouti. The authors' findings are similar to those of previous studies carried out in Ethiopia (15). But the results of the current study show a lower seroprevalence than that reported in other regions. For example, a seroprevalence of 28.9% was observed in northern Burkina Faso (16), 24.0% in Oman (17), 22.4% in Turkey (18), 19.0% in Côte d'Ivoire (19) and 10.0% in Ethiopia (20). This might be related to different husbandry systems and vaccination history. As these countries used to vaccinate small ruminants, whereas Djibouti did not, and serological tests do not differentiate between antibodies produced due to infection and those produced due to vaccination. The effect of husbandry and vaccination on the prevalence of PPR has been described by Lefèvre and Diallo and by Balamurugan *et al*. (1, 21).

The overall prevalence of antibodies to PPRV in small ruminants in Djibouti is higher in the regions of Tadjourah, Dikhil and Ali Sabieh (8.92%, 7.85% and 8.10%, respectively), than in the regions of Arta (3.80%) and Djibouti (1.28%). This could be linked to differences in the animal husbandry system used in the regions. The highest prevalence in those regions may be due to frequent migration of animals across the border owing to the fact that the nomadic movement of small ruminants from Djibouti to neighbouring countries in search of pasture and water during drought may increase the chances of contact with local sheep and goats, thereby facilitating the transmission of PPRV.

The high prevalence of anti-PPRV antibodies was observed in Tadjourah, Dikhil and Ali Sabieh could also be attributed to the high population of livestock. In the regions of Arta and Djibouti, farmers practise sedentary production, with limited movement of animals. This might have reduced contact between animals and thus minimised the transmission of the virus. Interestingly, the results for the Obock region, which was free of PPRV antibody in the present study, might

be related to the restriction of animal movement due to its geographical separation from its surrounding regions (Red Sea, Eritrea and mountainous borders), thereby restricting the introduction of the virus from infected regions.

The association of the prevalence of PPR with animal movement has been reported previously. For instance, Megersa *et al.* (20) observed higher prevalence in the Afar region of Ethiopia than the Gambella region, which was due to differences in the animal production system. Afar is characterised by pastoral production systems, where large-sized flocks are mixed with cattle and camels. In comparison, the Gambella region is more of an agro-pastoral region where small-sized flocks were kept separately (20). Furthermore, the current study's results are in line with the reports of Braide (22) which showed the transmission of PPRV to susceptible sheep and goat populations by migratory animals. The movement of animals, therefore, played an important role in the transmission and maintenance of PPRV in nature (23). Likewise, the results of Al-Majali *et al.* (24), showed that higher seroprevalence of PPR was recorded in regions with free animal movement than in other areas in Jordan. In addition to movement, limited fodder availability, especially during drought and lengthy dry seasons could have contributed to higher prevalence in those regions of Djibouti. Such nutritional deficiency can increase the susceptibility of animals to infection during periods of feed shortage, giving rise to the establishment of disease endemicity and year-round circulation of the virus with enhanced animal-to-animal contacts (25).

Species-specific prevalence of PPRV antibodies has been determined in this study. Goats were observed to have significantly ($p < 0.05$) greater prevalence of anti-PPRV antibodies (6.83%) than sheep (2.66%). Moreover, the results of the present study are consistent with other studies in Cameroon and Pakistan (26, 27), where a higher prevalence of PPR was reported in goats than in sheep. However, reports showing higher prevalence in sheep than goats are also documented elsewhere in the world (15, 16, 18).

The results of multivariable logistic regression showed that females were more likely to have anti-PPRV antibodies than males. It is evident that females had a significantly higher seroprevalence (6.68%) than their male counterparts (2.18%). This observation agrees with the findings of Waret-Szkuta *et al.* and Khan *et al.* (28, 29), who observed a significantly higher seroprevalence of PPR in females than males. This could be related to the physiological differences in which female animals are prone to infection as a result of the stresses related to production and reproductive activities (20). The authors' observations also agree with the findings of Luther *et al.* (30), who reported a significantly higher seroprevalence of PPR in females than in males. Male animals are not usually kept in a flock for a long period of time. They are often sold for meat at approximately one to two years of age, while females remain in the flock for breeding purposes. Thus, female animals have a greater exposure time than males in the flock. This may explain the higher seroprevalence obtained in females in this study.

Statistically significant difference ($p$ = 0.004) was observed in the prevalence of PPR among the age groups. Young small ruminants had a higher prevalence of PPR (8.25%) than adults (4.65%). The results of the current study were in agreement with the findings of Farougou *et al*. in Niger and those of Taylor *et al*. in Oman (17, 31), who observed a higher prevalence amongst young small ruminants than adults. This might be explained by the fact that the susceptibility of animals to infection by PPRV is associated with age (1). The observations of the present study, however, are not consistent with the results of Sow *et al.* (16) and Mahajan *et al.* (32) who reported higher seroprevalence in adults. This discrepancy could be due to the fact that the previous researchers sampled animals from vaccinated areas, while vaccination against PPR in Djibouti has never taken place.

## Conclusions

The present study revealed for the first time the occurrence of PPR in small ruminants in Djibouti, with low prevalence. PPR was shown to be widespread throughout all small ruminant producing areas of

Djibouti, except Obock. Of these five regions, three had high PPR prevalence, while the remaining two had low prevalence. The Government of Djibouti should therefore consider the results of this study in its plan to improve the small ruminants industry.

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**Table I**

**Results of seroprevalence of peste des petits ruminants in small ruminants in Djibouti**

| Variable | Number of samples examined | Number of positive samples (%) | 95% CI | $X^2$ | *p* value |
|---|---|---|---|---|---|
| **Regions** | | | | | |
| Obock | 157 | 0 (0.00) | 0–1.89 | | |
| Tadjourah | 314 | 28 (8.92) | 6.00–12.63 | | |
| Dikhil | 331 | 26 (7.85) | 5.19–11.29 | 27.47 | 0.001 |
| Ali-Sabieh | 321 | 26 (8.10) | 5.35–11.64 | | |
| Arta | 237 | 9 (3.80) | 1.75–7.08 | | |
| Djibouti | 156 | 2 (1.28) | 0.15–4.55 | | |
| **Species** | | | | | |
| Sheep | 301 | 8 (2.66) | 1.15–5.16 | 7.44 | 0.006 |
| Goat | 1,215 | 83 (6.83) | 5.47–8.39 | | |
| **Breed** | | | | | |
| Mixed | 491 | 28 (5.70) | 3.83–8.13 | 0.11 | 0.073 |
| Local | 1,025 | 63 (6.15) | 4.75–7.79 | | |
| **Sex** | | | | | |
| Male | 229 | 5 (2.18) | 0.71– 5.02 | 6.97 | 0.008 |
| Female | 1,287 | 86 (6.68) | 5.37–8.18 | | |
| **Age** | | | | | |
| Adult | 946 | 44 (4.65) | 3.39–6.11 | 8.14 | 0.004 |
| Young | 570 | 47 (8.25) | 6.12–10.81 | | |
| **Herd size** | | | | | |
| Small | 426 | 14 (3.29) | 1.80–5.45 | | |
| Medium | 629 | 50 (7.97) | 5.95–10.34 | 9.81 | 0.007 |
| Large | 461 | 27 (5.86) | 3.89–8.41 | | |
| **Production system** | | | | | |
| Sedentary | 175 | 3 (1.71) | 0.35–4.92 | | |
| Agro-pastoralism | 112 | 1 (0.89) | 0.02–4.87 | 16.04 | 0.001 |
| Pastoralism | 1,229 | 87 (7.079) | 5.71–8.65 | | |
| **Grazing** | | | | | |
| Zero-grazing | 123 | 2 (1.63) | 0.19–5.75 | | |
| Fenced | 84 | 1 (1.19) | 0.03–6.45 | 13.24 | 0.004 |
| Communal | 610 | 32 (5.25) | 3.61–732 | | |
| Migratory | 699 | 56 (8.01) | 6.11–10.22 | | |
| **Water point** | | | | | |
| Private | 207 | 5 (2.42) | 0.78–5.54 | 5.46 | 0.019 |
| Shared | 1,309 | 86 (6.57) | 5.28–8.05 | | |

CI: confidence interval

**Table II**

**Multivariable logistic regression analysis of the risk factors on the seroprevalence of peste des petits ruminants virus antibodies**

| Variable | Number of samples examined | Number of positive samples | Crude odds ratio (95% CI) | Adjusted odds ratio (95% CI) |
|---|---|---|---|---|
| **Species** | | | | |
| Sheep | 301 | 8 | 1 | 1 |
| Goat | 1,215 | 83 | 2.68 (1.28–5.61) | 2.95 (1.39–6.35)* |
| **Sex** | | | | |
| Male | 229 | 5 | 1 | 1 |
| Female | 1,287 | 86 | 3.20 (1.28–7.99) | 3.82 (1.51–9.67)* |
| **Age** | | | | |
| Adult | 946 | 44 | 1 | 1 |
| Young | 570 | 47 | 1.84 (1.20–2.81) | 2.29 (1.47–3.56)* |
| **Herd size** | | | | |
| Small | 426 | 14 | 1 | 1 |
| Medium | 629 | 50 | 2.54 (1.38–4.65) | 2.06 (1.10–3.83)* |
| Large | 461 | 27 | 1.83 (0.94–3.54) | 1.22 (0.60–2.44) |
| **Production system** | | | | |
| Sedentary | 175 | 3 | 1 | 1 |
| Agro-pastoralism | 112 | 1 | 0.51(0.05–5.02) | 1.02 (-3.84–1.79) |
| Pastoralism | 1,229 | 87 | 4.36 (1.36–13.96) | 1.77 (0.307–3.86) |
| **Grazing** | | | | |
| Zero-grazing | 123 | 2 | 1 | 1 |
| Fenced | 84 | 1 | 0.72 (0.06–8.16) | 0.98 (0.05–18.90) |
| Communal | 610 | 32 | 3.3 (0.79–14.16) | 0.55 (0.03–8.53) |
| Migratory | 699 | 56 | 5.26 (1.26–21.88) | 0.90 (0.05–15.41) |
| **Water point** | | | | |
| Private | 207 | 5 | 1 | 1 |
| Shared | 1,309 | 86 | 2.84 (1.13–7.08) | 0.70 (0.18–2.65) |

CI: confidence interval

*Statistically significantly

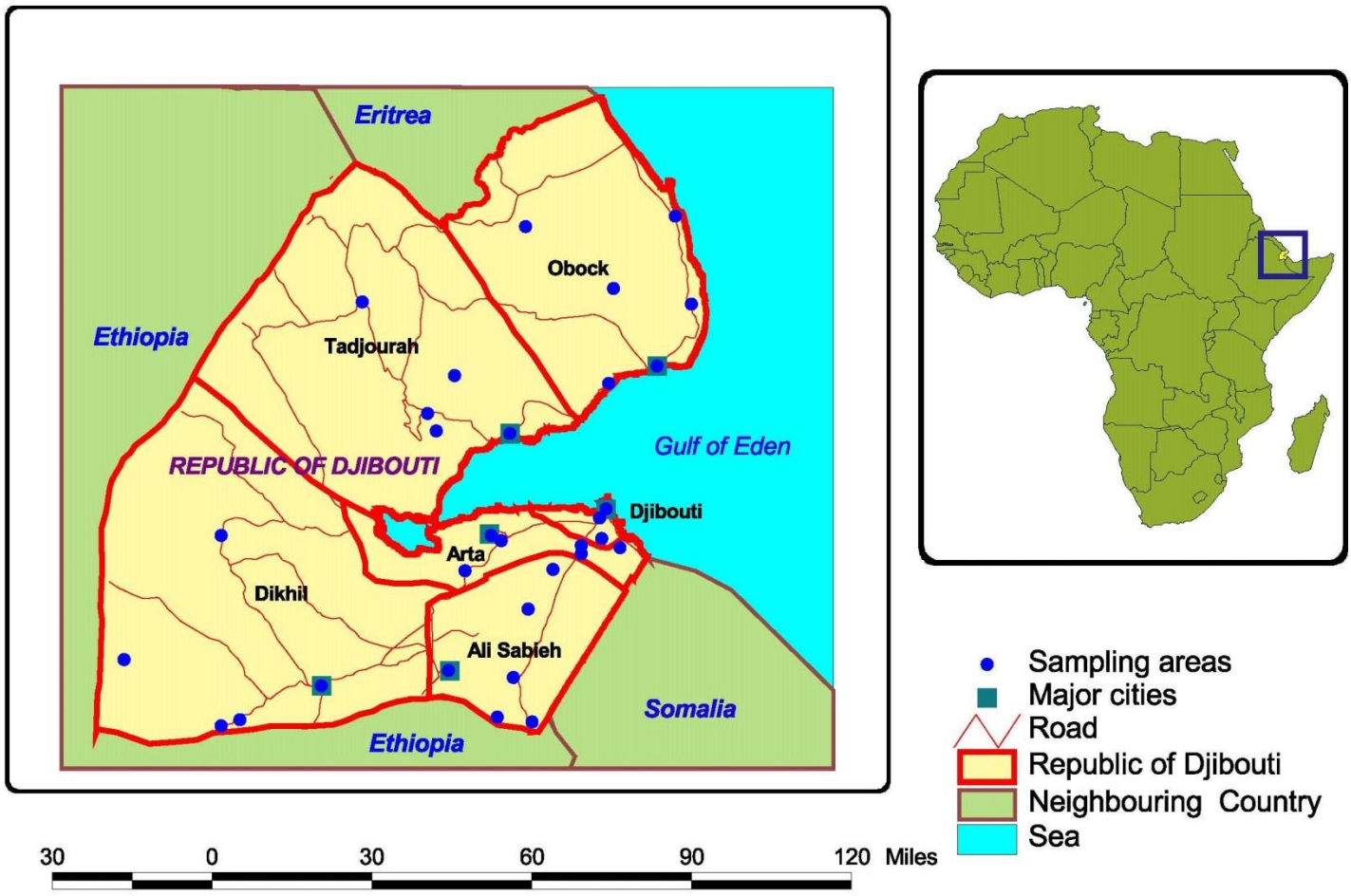


**Fig. 1**
**Map of Djibouti showing the sampling areas**