

DOMESTIC TRANSMISSION OF RIFT VALLEY FEVER VIRUS IN DIAWARA (SENEGAL) IN 1998

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Abstract. In 1998, circulation of the Rift Valley Fever (RVF) virus was revealed in Diawara by detection of IgM antibodies in sheep and isolation of the virus from mosquitoes caught outside a village. A seroprevalence study was carried out. Finger-prick blood samples, individual and collective details were obtained. One thousand five hundred twenty people (6 months - 83 years) were included. Overall prevalence in this group was approximately 5.2%. The prevalence in infants (6 months-2 years) was 8.5%. Age, gender, contact with a pond, presence of sheep, and abortion among sheep, and individual or collective travel history were not statistically associated with prevalence. Prevalence increased significantly when the distance to a small ravine, located in the middle of the village, decreased. The results suggest a low, recent, not endemic circulation of the virus. *Culex quinquefasciatus* was captured near the ravine. This mosquito, similar to *Culex pipiens*, can play a similar role in human-to-human transmission of the RVF virus.

INTRODUCTION

Rift Valley Fever (RVF) is a viral disease affecting livestock, especially sheep and goats, causing abortion in females and a high mortality rate in newborn animals. Humans can be infected directly by contact with blood or abortion products of infected animals, or indirectly by mosquito bites. Clinical symptoms include those of febrile illness, sometimes death, or other complications (retinitis, hepatitis, encephalitis, hemorrhages) (Abdel-Wahab *et al*, 1978; Meegan, 1979; Anonymous, 1988; Wilson *et al*, 1994).

During a major epidemic of RVF in the lower valley of the Senegal River in 1987, (Jouan *et al*, 1989) IgM prevalence reached 85% in domestic ruminants (Jouan *et al*, 1989). However, serosurveys conducted 1 to 3 years after the outbreak showed a progressive decrease in herd immunity in the Senegal River basin (Thiongane, 1994). In 1995 and 1996, an increase in IgM posi-

tive sheep and cattle was again observed along the Senegal River (Fontenille *et al*, 1998). In September and October 1998, RVF virus was isolated in Mauritania (Diallo *et al*, 2000). In November 1998, an entomological and veterinary study was carried out in the valley of the Senegal River to detect specific IgM antibodies in sheep, in the villages of Thillé Boubacar, Diawara and Kidira, isolate the virus in mosquitoes caught around a pond located in the vicinity of Diawara (Diallo *et al*, 2000). This latter village is located in Bakel's district, near the border of Mauritania and Mali.

A cross-sectional survey was carried out on the human population of Diawara to estimate the prevalence of RVF virus-specific immunoglobulin (Ig) G antibody in the population, to investigate a recent or previous circulation of the RVF virus and to identify the risk factors for exposure to the virus.

SUBJECTS AND METHODS

Study area

Diawara Village is located in Bakel district, in the region of Tambacounda, East Senegal. The

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expansion of the village to the north is limited by the Senegal River, which constitutes the border with Mauritania. The village is separated into two parts by a main pathway. In the northern, older part of the village, extended family compounds are small and close to each other. In the southern, newer part, the compounds are large and disseminated. The grasslands surrounding the village are flooded annually for a period of 3-6 months, after which they are extensively grazed by sheep and cattle. Around the most important of these temporary pools, mosquitoes infected with RVF virus were caught in 1998 (Diallo *et al*, 2000). Every rainy season, approximately a quarter of Diawara's population migrate to secondary compounds located in other villages, ten to fifteen kilometers from Diawara, to cultivate the land.

Population

Diawara's population is estimated at ten thousand people (extrapolated from the 1988 census) living in 301 compounds. Due to the lack of an exhaustive census of the population, a cluster sampling method was used. A cluster was a compound. Fifteen hundred people were necessary to insure the inclusion of 150 infants of 6 months to 2 years in the sample (10% of the population) so that we could estimate the prevalence in this age group with a precision of 5% (expected prevalence=5%, accepted alpha error=5%, estimated design effect=2). All compounds were eligible. The project protocol and the objectives were carefully explained to the assembled village population. With the informed consent of the head of the compound and of the individual (of the parents or legal guardians in case of children), all inhabitants living in a sampled compound, born or resident in Diawara before the first of January 1999 were included in the study. To prevent detection of maternally transmitted immunity in the infants, subjects under 6 months were not included. If the inhabitants of a sampled compound had already moved on in their secondary compounds, they were investigated at their new address. The heads of each sampled compound were interviewed using a general questionnaire to collect details (travel history, presence of sheep and abortion among those sheep, contact with the

pond where infected mosquitoes had been caught). An individual questionnaire was filled out for each subject to collect information regarding age, sex, and travel history in 1998. Collective and individual travel history were studied separately to collect further information about the place where people were exposed to the virus. Travel history in 1998 was thoroughly investigated for each subject, but travel was taken into account only if its duration exceeded 1 month. Collective travel corresponds to regular travel of a part of the population which occurs almost every year, during the rainy season and to the same destination (the secondary compound). Individual travel was to various destinations (except the secondary compound) taking place at any period in the year.

The protocol was approved by the ethics committee of the Pasteur Institute of Dakar and the Ministry of Health of Senegal.

Samples and laboratory methods

For the sake of specific immunoglobulin (IgG) antibody detection, finger-prick blood samples were collected on filter paper. If necessary, additional visits were performed to meet all eligible inhabitants of the compound. The samples were allowed to dry and stored in plastic boxes. In the Department of Virology at the Pasteur Institute in Dakar, the samples were tested by ELISA. The serum was eluted from the filter paper by soaking in the test diluent and diluted (PBS tween) to 1/400. The IgG test was performed with mouse anti-human IgG conjugate. Samples were considered positive if they had mean adjusted optical density values greater than a cut-off of 0.2 at 450 nm. This cut-off, used for many years by the WHO reference center for arboviruses in Senegal, has so far been higher than the mean value negative sample plus 3 standard deviations classically used as a cut off determinant and therefore appears to be appropriate for the studies carried out.

Statistical analysis

The "survey commands" of the statistical software STATA (Release 6.0. Stata Corporation) were used and the "compound" variable was specified as the primary sampling unit (PSU). The test of independence was the Pearson χ^2 sta-

tistic with the Rao and Scott second-order correction (Gordon *et al*, 1991; Gad *et al*, 1999). A test developed by O'Quigley and Schwartz (1986) permitted a comparison of the prevalence in different compounds, in the presence of low cell expectation. For multivariate analysis, logistic regression of the survey data was used. All variables which p-values below 0.40 were used in the first model. A backward stepwise method was chosen to obtain the final model, including only relevant variables.

RESULTS

The study took place between July 12-29, 1999. Of the 77 compounds sampled, 3 were permanently unoccupied, 2 were annexed to visited compounds. In one of these, we could not reach the inhabitants in their secondary compound, and in 6 (7.8%), the heads of the compounds did not agree to participate in the study. In the 65 compounds visited, 1,579 people were eligible, but 47 (3%) refused blood sampling, and for 12 subjects, serologic data are not available.

In total, 1,520 people were included in the study (54% females), age 6 months to 83 years. The median age was 14 years.

The global prevalence of RVF virus was estimated at 5.2% (79/1,520) with a confidence interval of 3.7-7.0%. The prevalence in infants was 8.5%, with a confidence interval of 4.4-15.9%. The global design effect, calculated as the ratio of the cluster sample variance and the randomized sample variance, was 2.05.

Effect of age and gender

Prevalence was similar in all age groups ($p=0.73$) (Table 1) and in the two genders (4.9% for women and 5.5% for men) ($p=0.58$). It was not possible to identify, in each compound individuals, involved in slaughtering domestic animals because this is a collective activity. All adult men are included in the slaughter of animals, children of both gender take a part in the skinning and butchering and women cook the meat. No difference of prevalence was observed between children under 10 years (5.8%), women (5.2%), and men (5.6%) ($p=0.60$).

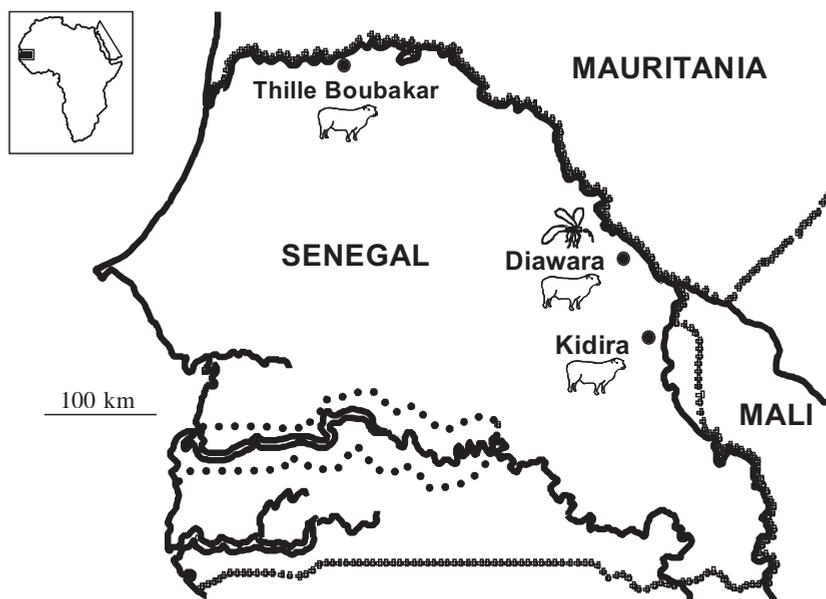


Fig 1—Map of Senegal and surrounding countries. Sheep point out the 3 villages where RVF specific IgM have been detected in sheep (Thille Boubakar, Diawara and Kidira) and the mosquito indicates that the RVF virus has been isolated in mosquitoes caught near Diawara.

Table 1
Reported characteristics and activities and distribution of anti-RVF virus Immunoglobulin (Ig) G prevalence among 1520 inhabitants of Diawara, in July 1999.

Variables	N ^o	N ^o of IgG+	IgG Prevalence		P ^a
			%	CI 95%	
Age ^b					
6-11 months	37	4	10.8	4.1 - 25.4	0.73
1 year	21	2	9.5	2.4 - 31.4	
2 years	36	2	5.6	1.3 - 20.8	
3 years	48	2	4.2	1.0 - 16.4	
4 years	56	4	7.1	2.8 - 17.1	
5-9 years	285	14	4.9	2.3 - 10.1	
10-14 years	304	9	3.0	1.6 - 5.5	
15-19 years	211	12	5.7	3.1 - 10.3	
20-29 years	152	10	6.6	2.7 - 15.3	
30-39 years	126	5	4.0	1.7 - 9.0	
40-49 years	87	6	6.9	3.0 - 15.1	
50-59 years	71	4	5.6	2.2 - 14.0	
≥ 60 years	83	4	4.8	1.8 - 12.1	
Gender					
Female	816	40	4.9	3.2 - 7.4	0.58
Male	704	39	5.5	3.9 - 7.9	
Collective travel history*					
Yes	290	18	6.2	3.1 - 12.1	0.58
No	1,040	52	5.0	3.3 - 7.5	
Individual travel history*					
Yes	208	11	5.3	3.2 - 8.6	0.86
No	1,040	52	5.0	3.3 - 7.5	
Presence of sheep					
Yes	1,373	68	5.0	3.5 - 6.9	0.36
No	147	11	7.5	3.2 - 16.4	
Abortion among sheep					
Yes	159	8	5.0	2.8 - 8.9	0.91
No	1,361	71	5.2	3.7 - 7.3	
Contact with the pond					
Yes	959	43	4.5	2.9 - 7.0	0.26
No	561	36	6.4	4.1 - 10.0	
Total	1,520	79	5.2	3.8 - 7.1	

^ap-value of the Pearson χ^2 statistic with the Rao and Scott (1981, 1984) second-order correction

^b3 missing data

Effect of collective or individual travel history

Collective travel is generally longer (median duration 5.8 months) than individual travel (median duration 3.4 months). Among the 381 inhabitants who usually move collectively to their secondary compounds, 290 (76%) did actually have a collective travel history in 1998. Two hundred and eight subjects had an individual travel

history in 1998 and 21 people had both collective and individual travel history in 1998. For 3 people who moved in 1998, the destination could not be specified. In comparison with people that didn't move at all in 1998 (1,040 subjects), no variations of prevalence were found with people who had collective ($p=0.58$) or individual ($p=0.86$) travel history.

Table 2
Distribution of the 65 compounds in the prevalence groups and mean distance to the gully, Diawara 1999.

Prevalence group	N° of compounds	Mean distance (meter) ^a	Median distance (meter) ^a
0%	26	162.6	148.0
0.01% - 5%	15	129.3	124.0
5.01% - 10%	15	122.0	99.0
>10%	9	75.1	64.0

^a Estimated from the distances measured on the map of the village, between center of the compounds and middle of the gully.

Table 3
Estimation of a backward selection model for anti-FVR virus IgG seropositivity among 1,517 Diawara inhabitants (logistic regression for survey data).

	Model 1		Model 2		Model 3		Final model	
	OR ^a	p						
Distance (meter)	0.990	0.000	0.990	0.000	0.990	0.000	0.990	0.000
Contact with the pond	0.773	0.286	0.770	0.281	0.755	0.233		
Presence of sheep	0.864	0.680	0.867	0.687				
Age (year)	1.003	0.688						
Log likelihood	-296.2		-296.28		-296.36		-297.06	
lr.test ^b	df ^c		1		1		1	
	p		0.69		0.85		0.63	

^a : Odds ratio; ^b : likelihood-ratio test; ^c : degree of freedom

Effect of the presence of sheep and abortion among the sheep

Sheep were present in 82% (53/65) of the compounds according to observation of sheep during the visit of the compound and questionnaire answering of the head of the compound. The 12 compounds without sheep are located in the northern part of the village, where houses are close to each other. Prevalence of Ig G specific for RVF virus is higher (7.5%) in the 12 compounds where no sheep was present than in the others (5.0%). According to the responses of the inhabitants, abortions of sheep were noticed only in 8 compounds. Prevalence in these compounds (5.0%) was similar to prevalence in the other compounds (5.2%) ($p=0.91$).

Effect of the contact with the pond

Inhabitants living in the northern and west-

ern parts of the village have rare contact with the pond but frequent contact with the river. In contrast, in other parts of the village, almost all the inhabitants have contact with the pond to take water, to fish, to look for forage or just for fun. Despite the fact that RVF virus infected mosquitoes had been caught around the pond, prevalence found in the compounds where inhabitants have contacted with the pond (4.5%) was lower than in the other concessions (6.4%).

Effect of the compound

Prevalence of the 65 compounds was statistically heterogeneous ($p<0.003$). Using the Poisson distribution law, five compounds with high prevalence were identified. On the map of the village, produced by the Cartography Department of the National Statistic's Direction, these five compounds were clustered around a gully,

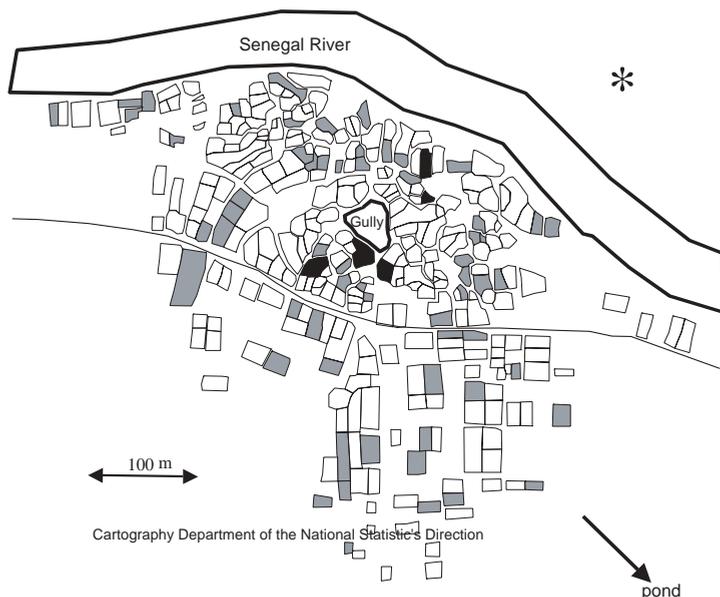


Fig 2—Map of the village of Diawara. Boxes represent the compounds, black boxes are the compounds with high prevalence and the grey ones are the other visited compounds. The black line represent the main pathway.

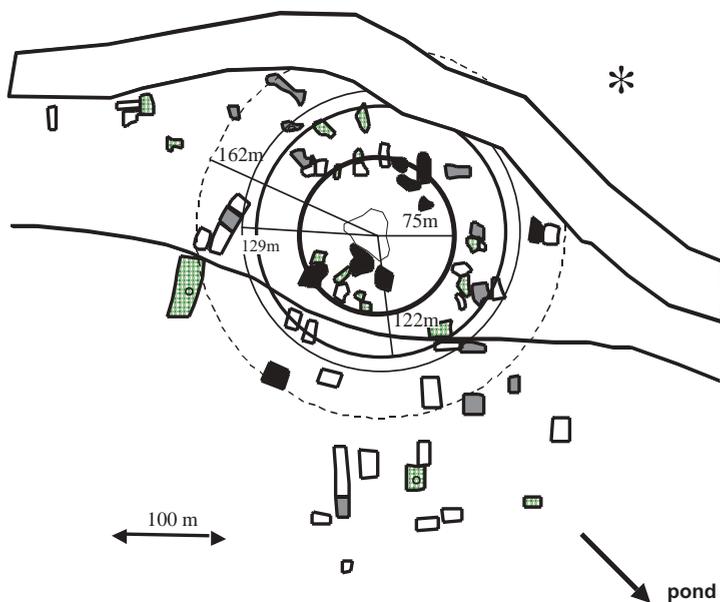


Fig 3—Map of the village presenting only the visited compounds, classed into 4 prevalence (Pv) groups (black boxes (group 1): $P_v > 10\%$; diagonal grid boxes (group 2): $5\% < P_v \leq 10\%$; dotted boxes (group 3): $0\% < P_v \leq 5\%$ and white boxes (group 4): $P_v = 0\%$). The thick, medium, thin and dotted circles represent respectively the median distances of the prevalence group 1, 2, 3 and 4 to the gully.

a small ravine, located in the northern part of the village (Fig 2). Regarding this result, the relation between prevalence and distance to the gully was investigated further. The distance be-

tween the 65 compounds and the gully was measured on the map. The prevalence found in the compounds increased significantly when the distance to the gully decreased ($R = -0.43$,

$p < 0.001$, Spearman rank correlation). To illustrate this relation on the map of the village, prevalence (Pv) was classed into 4 groups: group1, $Pv = 0\%$; group2, $0\% < Pv \leq 5\%$; group3, $5\% < Pv \leq 10\%$; group4, $Pv > 10\%$. The geographical distribution of these 4 groups was roughly concentric (the highest prevalence group, in the middle and the lowest, on the edge) and the center of this target was the gully (Fig 3, Table 2). A significant association between prevalence classes and mean distance to the gully was found again ($p = 0.006$, Kruskal-Wallis test).

Multivariate analysis

The variables "distance", "contact with the pond" and "presence of sheep", of which p-value was below 0.40, were introduced in the first model. The quantitative variable "age" was forced into this model owing to its interest in the study. The variables "age", "presence of sheep" and "contact with the pond" were successively removed as they were not relevant to explain the data (Table 3). The final model only contained the variable "distance".

DISCUSSION

Anti-RVF virus IgG global prevalence was estimated at 5.2% and prevalence for infants at 8.5%. No variation of prevalence was found for the following factors: age, gender and individual or collective travel history. This study did not bring out the classic risk factors (contact with sheep or abortion among sheep) or the expected risk factor (contact with the pond where infected mosquitoes had been caught). But it stressed the role of the vicinity to a gully, located in the middle of the village, as a risk factor.

Previous studies performed in Senegal stressed the absence of cross-reactivity when using immunocapture ELISA with phleboviruses previously isolated in West Africa, namely Gabeck Forest, Saint-Floris or Gordil (Zeller, 1997). The relevance of the exclusion of infants under 6 months is further confirmed by the observation of 4 RVF IgG positive infants whose mothers were negative, with the exception of one.

The fact that global prevalence differs from zero shows that the RVF virus has circulated in

Diawara inhabitants. The similar prevalence found with and without collective travel history tend to indicate that the circulation of the virus took place in Diawara. Global prevalence found in this study is comparable to prevalence found in 5 to 15 year old children in Barkedji (in northern Senegal, 450 km west of Diawara) in 1993 (Zeller, 1997). It is higher than prevalence among the overall population ($< 3\%$) in southeastern and southwestern Senegal (Zeller, 1997) but far lower than prevalence observed in the semi-nomadic Peul people (22.3%) living in the rural settlement of Yonofere, in north-central Senegal (75 km east of Barkedji) (Wilson *et al*, 1994). It is comparable to results reported by serologic surveys, in the absence of human disease, in surrounding countries (Mali, Guinea) and in numerous African countries (Sudan, Nigeria, Gabon, Angola, Botswana, Central African Republic and Kenya (Saleh, 1981; Johnson *et al*, 1983; Wilson *et al*, 1994). The presence of IgG antibodies in the age group, six months to two years, matches up with both a recent or an endemic circulation of the virus. But the similar prevalence in all age groups disagrees with a long-term exposure in a situation of endemic transmission. The seroprevalence study carried out 13 years after a major outbreak, in the epidemic context of the Nile river delta of Egypt, provided similar results (Corwin *et al*, 1993). In contrast, in the endemic context of Yonofere, prevalence increased with age in both genders (Wilson *et al*, 1994).

This study did not bring out the direct transmission risk factors, such as contact with sheep or abortion among sheep, raised in other studies. In fact, direct transmission has been stressed in two different situations: in association with a high RVF virus seroprevalence in domestic animals [high level endemic transmission, (Wilson *et al*, 1994) or major outbreak] (Anonymous, 1994), and in association with a particular occupation (handling of carcasses in abattoir workers) (Abu-Elyazeed *et al*, 1996). But even in these situations, mechanisms of transmission remain unclear. In Yonofere, despite of high IgG prevalence in both sheep (40%) and humans (37%), there was no relation between seroprevalence in sheep and humans living in the same compound and there was no evidence of clustering

within compounds or huts (Wilson *et al*, 1994). In Kenya, as well, the importance of direct transmission seems to be limited. Despite frequent widespread epizootics of RVF in ruminants, the level of RVF antibody is low in the population, even in the nomadic pastoralists (Johnson *et al*, 1983). The frequency of contacts between human and infected domestic animals is often put forward to explain the dissimilar results found in different populations and countries. However, they could be determined by the distribution, abundance and behavior of RVF vectors, which are not always thoroughly investigated.

This study was mainly motivated by the isolation of RVF virus from *Culex poicillipes*, a mosquito which fed on humans, birds, bovine, ovine and other animals (Chandler *et al*, 1976; Gordon *et al*, 1991), and which was recently identified as a new RVF vector in nature (Diallo *et al*, 2000). The hypothesis that *Cx. poicillipes*, occurring near the pond, played the role of bridge vector between animal and human was not confirmed in this study. Geographical distribution of prevalence groups disagrees with the involvement of a vector living outside the village in human infection. Regarding the results of the epidemiological study, few hypotheses about the causal factors were proposed, such as slaughtering or elimination of sick animals in and around the gully (Turell *et al*, 1990). The most likely hypothesis was the presence of a potential RVF vector breeding in the water of the gully. Till this epidemiological study, entomological investigations were only carried out outside the village. During the following rainy season, mosquito captures were also performed around the gully. Results showed that *Cx. poicillipes* was scarce inside the village where water is often corrupted by the surrounding houses and that the predominant species belonged to the *Culex pipiens* complex. According to the subtropical location of Senegal, this species was identified as *Culex quinquefasciatus* Say. This mosquito normally breeds in water rich in organic materials (Roberts, 1996), and thus is well adapted to the human environment (Edwards, 1941). It is very close to *Cx. pipiens* Linnaeus, the second species of the *Cx. pipiens* complex (Urbanellim *et al*, 1995), and the major vector involved in the

RVF epidemic of Egypt in 1977 (Hoogstrall *et al*, 1979; Meegan *et al*, 1980). As it feeds readily on humans and is often predominant in indoor and human bait collections, it is considered as the principal vector for human-to-human transmission in Egypt (Turell *et al*, 1996; Gad *et al*, 1999). Could *Cx. quinquefasciatus* have played the same role in Diawara in 1998? No virus was isolated from the 920 *Cx. quinquefasciatus*, caught around the gully. But these virological results do not deny the hypothesis of a human-to-human transmission performed by *Cx. quinquefasciatus* as (i) entomological investigations have been carried out two years after detection of the virus circulation (in July and October 2000), and (ii) no circulation of RVF virus could be detected inside and around Diawara in 2000 (no isolation of the virus from the 26,361 mosquitoes and no sheep with specific IgM). It is important to confirm or deny that hypothesis by carrying out complementary studies in big villages or small towns because confirmation of the presence of mosquitoes able to transmit RVF virus and to live in the domestic environment could have important implications in the epidemiological and entomological control of this disease in a large part of Africa.

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