Risk associated with bovine brucellosis in selected study herds and market places in four countries of West Africa

Animal Health Working Paper 2

.

Fred Unger

Susanne Münstermann

Alphonse Goumou

Correia Nunes Apia

Mamady Konte

Michaela Hempen

ITC

International Trypanotolerance Centre
P.O.Box 14, Banjul, The Gambia

Authors' affiliation

Fred Unger, International Trypanotolerance Centre, P.M.B. 14,
Banjul, The Gambia
Susanne Münstermann, International Trypanotolerance Centre, P.M.B. 14,
Banjul, The Gambia
Alphonse Goumou, Departement National d´ Èlevage, Conakry, Guinée
Correia Nunes Apia, Direcção Geral da Percuária, CP 26, Bissau, Guiné Bissau
Mamady Konte, ISRA, BP 3120, Dakar, Senegal
Michaela Hempen, International Trypanotolerance Centre, P.M.B. 14,
Banjul, The Gambia

Working papers contain results of research done by ITC scientists, consultants and collaborators. The author(s) alone is (are) responsible for the contents.

ISBN 9983 9910 20

Correct citation: F. Unger, S. Münstermann, A. Goumou, C. N. Apia, M. Konte, M. Hempen A., 2003.
Risk associated with bovine brucellosis in selected study herds and market places in 4 countries of West Africa.
Animal Health Working Paper 2. ITC (International Trypanotolerance Centre), Banjul, The Gambia, 37 pp.

LIST OF CONTENT

ACKNOWLEDGEMENTS

The authors wish to thank **Dr Karsten Nöckler** and **Dr Annemarie Kaesbohrer**, Institute for Risk Assessment (BfR), Reference Laboratory for Brucellosis, Berlin (Germany) for scientific editing of this publication. The assistance of the field technicians in all countries is gratefully acknowledged. The advice given by the Department for International Animal Health, Freie Universität Berlin (Germany) is particularly recognised. Our thanks go to Dr Justin Akakpo, EISMV, Dakar (Senegal) for the training given on CFT and milk-ELISA. Dr Jakob Zinsstag`s, Swiss Tropical Institute, Basel (Swizerland) assistance on providing the milk-ELISA is greatly appreciated. Funding of this study was provided by the EC funded PROCORDEL project (EDF 7, 6157 REG) and by the Senate of Berlin, Germany.

SUMMARY

Zoonoses are of particular public health importance in societies that live closely together with their livestock. Brucellosis, in particular, is easily transmitted via raw milk, the predominant consumption pattern for this commodity in West Africa.

In order to assess the importance of brucellosis, 5538 cattle in selected districts supplying milk to the local markets in Guinea (Dubréka, 17 farms; Boké, 19 farms; Coyah, Dubréka and Labé each 18 farms), The Gambia (Central River Division, 20 farms), Guinea Bissau (Bafata, 15 farms; Gabu 9 farms) and Senegal (Bassin Arachidier, 30 farms) were enrolled in a bovine brucellosis serological herd screening survey (May 2001 – June 2003). All serum samples were screened using Rose Bengal Plate Test (RBT) and Complement Fixation Test (CFT) as confirmatory test for RBT positive samples. Bulk milk samples were collected on some farms and subjected to Milk Ring Test (MRT) or ELISA test (BOMMELI®). Farmers were interviewed on their knowledge and awareness of brucellosis.

Results indicate different epidemiological situations for brucellosis in the four countries. Highest mean prevalences of all animals per area surveyed were found in the District of Bafata (Guinea Bissau) with 18.6 %, followed by three Districts of Guinea, Dubréka (12.7 %), Boké (6.3 %) and Coyah (5.9%). In Gabu (Guinea Bissau) 5.7 % and Forehcariah (Guinea) 3.8 % prevalences were reported. These values were considerably lower in The Gambia (1.1 %) and in Senegal (0.6 %) and the District of Labé in Guinea where the disease was absence. Highest herd prevalences were also demonstrated for Bafata (Guinea Bissau) with all sampled herds (n= 15) testing at least one animal positive. In Dubréka 16 of 17 herds, Coyah 15 of 18 herds, Gabu 7 of 9, Boké 14 of 19 herds and Forehcariah 11 of 18 herds were reported positive. In contrast, 2 of 20 herds were found positive in The Gambia and 3 of 30 in Senegal.

All herds tested positive in their bulk milk samples (MRT or ELISA) were always confirmed by a positive CFT result of at least one animal in the herd, but not all serologically positive herds were identified by bulk milk samples.

The knowledge of farmers on the zoonotic character and the ways of transmission of the disease in cattle and man was generally poor. However, farmers knowledge seems to be better in Guinea Bissau. With the exception of some farms in Guinea Bissau milk was consumed non-heated.

Cases of suspected brucellosis-like infections in man, all of them found on serologically positive farms, were reported during interviews in Guinea (n= 7) and Guinea Bissau (n= 5). High prevalences in cattle in Guinea prompted testing of volunteers (herdsmen and herd owners) in the same districts (Dubréka) with seven of 20 people diagnosed positive. The results in man underline the high public health risk associated with bovine brucellosis in Guinea and Guinea Bissau.

1 INTRODUCTION

Zoonoses are of public health importance through direct infection or consumption of contaminated animal products and are an occupational hazard. With increasing demand for milk and derived products the possible spread of milk-borne diseases also increases. This is particularly the case for brucellosis, causing severe disease symptoms in humans. 500,000 cases of human brucellosis are estimated to occur worldwide every year (ANON, 1986; HAHN, 1991). Particularly in developing countries prevalence rates in man are often high, because of close contact to animals and traditional food consumption customs (NICOLETTI, 1984). However, there is a lack of good data on the occurrence and impact of zoonotic infections, including brucellosis in many developing countries (PERRY et al., 2002, Mc DERMOTT and ARIMI, 2002). In tropical Africa *B. abortus* seroprevalence rates among the cattle population of up to 40%, and in some areas even higher, are reported (AKAKPO and BORNAREL, 1987; O.I.E., 1987). The disease has considerable impact on the economy through loss of milk and meat, restrictions in international livestock trade and by diminished animal working power.

Human brucellosis is known as a disease presenting a variety of symptoms, affecting multiple systems and causing different forms of localised infection. It is characterised by a febrile reaction and usually associated with splenomegalie and osteoarticular manifestations. Further complications are hepatitis, endocarditis, orchitis and meningoencephalitis (DJORDJEVIC, et al., 2003; MASOURIDOU et al., 2003). In particular in developing countries diagnostic difficulties exist, since the main symptoms (fever and back/joint pain) are often similar to other widespread diseases like malaria and specific tests are not available.

Measures against brucellosis aim at the control and, if possible, the eradication of the agent in the animal reservoir. As the disease often goes undetected the identification of infected herds and animals is of prime importance.

This investigation on brucellosis prevalence was carried out in the framework of a regional study in four countries of West Africa (The Gambia, Senegal, Guinea Bissau and Guinea). Nearly 100 % of milk produced in the region is consumed raw or fermented, hence is an ideal medium for the spread of this zoonosis. Brucellosis is endemic in many west-African countries but data on the prevalence of brucellosis are limited and if available often not representative or up to date.

The latest information for Guinea dates from 1997 and it indicates a national average prevalence of 3.5 %. There are, however, some districts with infection rates of up to 16.7% (CONDE, 2000). For Guinea Bissau infection rates of 40% are reported for the district of Bafata (DGP, 1996). However, the data for Guinea and Guinea Bissau are based on non-representative sampling. For the Bassin Arachidier (Senegal) an average prevalence of 8.9 % was stated in 1994 by KONTE. In The Gambia no data are available for the last 20 years.

In order to assess the sero-prevalence of brucellosis in cattle several pilot studies were carried out between May 2001 and June 2003 in Senegal, The Gambia, Guinea and Guinea Bissau in form of on-farm screenings.

The selection of study sites for each country was based on criteria like high cattle density, supply of milk to urban markets and high reported prevalences for brucellosis in previous studies. In The Gambia the southern Central River Division (CRD south) and in Guinea Bissau Gabu and Bafata were selected as areas with known high cattle density. In the Bassin Arachidier of Senegal and 3 selected districts of Guinea (Coyah, Dubréka, Forehcariah) the criteria was the supply of milk to urban markets. Labé and Boké in Guinea were selected because of previous reports on high brucellosis prevalences (CONDE, 2000, GOUMOU, 2002).

2 MATERIALS AND METHODS

During the study period of May 2001 to June 2003 a total of 164 herds from Guinea, Guinea Bissau, Senegal and The Gambia were enrolled in a herd screening survey. An overview on the study sites, period, sample sizes and tests applied is given in table 1.

Table 1: Study sites, period and sampled herds

Country	Area	Period	No. herds examined	Applied test on serum	Applied test on bulk milk
Guinea	Dubréka	May - Aug 2001	17	Rose Bengal Plate test (RBT) followed by Complement Fixation (CFT) of positive reactors	ELISA
	Boké		19		ELISA
	Labé	Mar - Oct 2002	18		-
	Coyah		18		-
	Forehcariah		18		-
Guinea Bissau	Bafata	May 2002 – Jun 2003	15		ELISA
	Gabu		9		-
The Gambia	CRD south	May - Oct 2001	20		Milk Ring Test
Senegal	Bassin Arachidier	Jun 2001 – Jan 2002	30		Milk Ring Test

Boké district is located in northwest Guinea, bordering Guinea Bissau. Boké, the District capital, is 300 km away from Conakry. Dubréka, Coyah and Forehcariah districts are situated close to Conakry. The distance to Conakry ranges between 50 - 100 km. Milk produced in these districts is therefore sold also at Conakry markets. Bafata is located in the centre and Gabu in the western part of Guinea Bissau, bordering Guinea. The distance from Bafata to Bissau is 120 km and from Gabu to Bissau 180 km, respectively. Milk produced in Bafata is sold also at Bissau markets. Bafata and Gabu represent 75% of the cattle population of the country. The CRD south is situated in the western part of The Gambia and is characterized by its high cattle population. Milk is sold only locally. The Bassin Arachidier (Senegal) is located southwest of Dakar bordering the northern frontier of The Gambia. Milk produced in the region is sold at the big urban markets (i.e. Kaolack and Fatick). The region is characterized by its on-going transition from the extensive traditional husbandry to the market orientated system.

The cattle population in Guinea, Guinea Bissau and The Gambia consists of trypanotolerant N´Dama kept in extensive traditional husbandry system. In the Bassin Arachidier of Senegal the predominant cattle breed is the Zebu with an increasing proportion of introduced crossbred cattle. The majority of cattle in the region are on transhumance during March to June.

All study herds were selected by stratified random sampling from a larger sampling frame assuming a herd prevalence of at least 15% and a 95% confidence interval (THRUSFIELD, 1995). The criteria for selection of herds was the supply of milk to two local markets. On herd level only cattle older than 6 months were sampled. Assuming a prevalence of at least 10% (95 % confidence interval) a respective number of animals (according to herd size) were selected randomly and stratified by age on herd base (THRUSFIELD, 1995). In a subsequent step, bulk milk samples (100-500ml) were collected from the selected herds**.**

Blood samples were tested using Rose Bengal Plate Test (RBT). Clear agglutination reactions were regarded as positive. Positive samples were kept at –20 ºC and later subjected to Complement Fixation Test (CFT) according to OIE Standards (OIE, 2001). *B. abortus* antigen, positive and negative controls as well as the test reagent for the RBT were produced and provided by the Institute for Risk Assessment (BfR), Reference Laboratory for Brucellosis, Berlin (Germany). Complement, VBD buffer and Amboceptor was supplied by VIRION/SERION GmbH, Würzburg (Germany). The haemolytic system was always freshly prepared prior to use. CFT was performed as cold fixation. All sera were inactivated at 57 ºC for 30 min. Sera with anti-complementary activity were inactivated at 60 ºC for the same time and retested.

Bulk milk samples collected in The Gambia and Senegal were tested for brucellosis using Milk Ring Test (MRT). Positive and negative controls as well as the test reagent for the MRT were produced and provided by the BfR, Berlin (Germany). In addition

37 bulk milk samples collected in Guinea and Guinea Bissau were stored at –20 ºC and subjected to the milk ELISA (BOMMELI®, Switzerland).
Based on of CFT results three prevalence rates were calculated as follows:

- Herd prevalence: $\frac{\text{number of herds with at least one positive reactor}}{\text{number of herds sampled}}$

- Within-herd prevalence: $\frac{\text{number of positive reactors}}{\text{number of serum samples tested from this herd}}$

- Individual animal prevalence: $\frac{\text{number of individual positive reactors}}{\text{number of serum samples tested.}}$

The rate of confirmation of positive RBT by CFT was calculated (number of CFT reactors/number of positive RBT samples).

Using results of the MRT and milk ELISA results, herd prevalence rates for brucellosis-contaminated milk was calculated as described above.

Herd managers were interviewed using a semi-structured standardised questionnaire on the current and past occurrence of brucellosis-like disease and their general knowledge on zoonoses.

In an attempt to get indicative information on human brucellosis in a high prevalence area for this disease in cattle in Guinea, where milk is consumed always untreated, blood samples from risk group volunteers (herder, herd owner and veterinarians) were taken in a local health centre in Dubréka. Twenty volunteers were selected randomly by the DVO from villages around the health centre. RBT and CFT were used as diagnostic tests.

3 RESULTS

3.1 Study population

Details on the study populations for all four countries screened for brucellosis are summarized in Tab 2.

Table 2: Study populations in the four countries

Country/Area	No. herds	No. bulk milk samples	No. of animals sampled			
			Total	Cows and heifers	Bulls	Calves
Overall	**164**	**87**	**5538**	**3926**	**1100**	**512**
Guinea	**90**	**22**	**3861**	**2658**	**743**	**460**
Dubréka	17	10	749	590	103	56
Boké	19	12	675	501	112	62
Labé	18	-	810	567	166	77
Coyah	18	-	810	549	133	128
Forehcariah	18	-	817	451	229	137
Guinea Bissau	**24**	**15**	**733**	**571**	**135**	**27**
Bafata	15	15	539	428	95	16
Gabu	9	-	194	143	40	11
The Gambia						
CRD south	20	20	465	324	133	8
Senegal						
Bassin Arachidier	30	30	479	373	89	17

During May 2001 to June 2003 a total of 5538 blood samples from 164 herds were taken in the four countries. In addition, 87 bulk milk samples were collected in Senegal, The Gambia and from some herds in Guinea (Boké and Dubréka) and Guinea Bissau (Bafata).

3.2 Confirmation rate in CFT for RBT positive samples

All blood samples were screened using the Rose Bengal Plate Test. 449 out of 5538 (8.1 %) reacted positive and were subjected to the Complement Fixation Test for confirmation. 74.2 % (333/449) of positive sera in RBT were confirmed by CFT. The confirmation rate differed between the countries as shown in table 3.

Table 3: Confirmation rate in CFT for RBT by country

	Overall	**The Gambia**	**Senegal**	**Guinea**	**Guinea Bissau**
Total	**5538**	**465**	**479**	**3861**	**733**
+*ve* in RBT	449	5	3	300	141
+*ve* in CFT	333	5	3	214	111
Confirmation rate (%)	**74.2**	**100**	**100**	**71.3**	**78.7**

3.3 Individual animal and herd prevalence

Individual animal prevalence and *herd prevalence* based on CFT for all regions are presented in Table 4.

Table 4: Individual animal and herd prevalences based on CFT stratified by region and country

Region Area	No. of animals	Individual +*ve* reactors	Individual animal prevalence in % (CI)	No. of herds	Herds +*ve*	Herd prevalence rate in % (CI)
Guinea	**3861**	**214**	**5.5^{1} (4.8,6.3)**	**90**	**56**	**62.2^{1} (59.3,78.5)**
Labé	810	0/810	0^{a}	18	0	0^{a}
Boké	675	42/675	6.3^{b} (4.5,8.1)	19	14	73.7^{b} (53.9,93.5)
Dubréka	749	95/749	12.7^{c} (10.2,15.2)	17	16	94.1^{b} (82.9,100)
Coyah	810	48/810	5.9^{b} (4.3,7.5)	18	15	83.3^{b} (62.6,100)
Forehcariah	817	29/817	3.8^{b} (2.5,5.1)	18	11	61.1^{b} (38.6,83.6)
Guinea Bissau	**733**	**111/733**	**15.1^{2}** (12.5,17.7)	**24**	**22**	**91.6^{2} (80.5,100)**
Bafata	539	100/539	18.6^{a} (15.3,21.9)	15	15	100
Gabu	194	11/194	5.7^{b} (2.4,8.9)	9	7	77.8 (50.6,100)
The Gambia						
CRD south	465	5	**1.1^{3} (0.2,2.0)**	20	3	**15^{3} (0,30.6)**
Senegal						
Bassin Arachidier	479	3	**0.6^{3} (0,1.3)**	30	3	**10^{3} (0,20.7)**

Superscript letters: Significant differences between districts within a country
Superscript figures: Significant differences between countries

Individual animal prevalences (IAP) differed between and within the countries. Significant higher overall IAP´s were found in Guinea Bissau (15.1%) compared to all other countries. Overall IAP´s reported for Guinea (5.5%) were also significantly higher than those reported for Senegal (0.6%) and The Gambia (1.1%). In Guinea, Dubréka (12.7%) had significant higher prevalences than all other districts, whereas differences reported for Boké (6.3%), Coyah (5.9%) and Forehcariah (3.8%) were significantly higher compared to Labé where no positive case was found. With 18.6% brucellosis was more frequent in Bafata (Guinea Bissau) than in Gabu where 5.7% were reported.

The overall brucellosis *herd prevalence* rate (HP), i.e. at least one positive CFT reactor identified in a herd, differed between the countries with significantly higher values reported for Guinea Bissau (91.6%). In addition HP´s were significantly higher in Guinea (62.2%) than in The Gambia (15%) and Senegal (10%).

3.4 Individual animal prevalence and age

Guinea and Guinea Bissau

Prevalences for Guinea and Guinea Bissau increased with age of cattle (Tab 5). As no positive reactor for brucellosis was found in Labé (Guinea) this district was excluded from the calculation.

In Guinea 14 % (81/566) of CFT positive cattle were older than 6 years, 11 % percent (63/575) 3 – 6 years and 4.1 % (62/1527) 1 – 3 years. In calves 2.1 % reacted positive (8/383). From the age related differences in prevalences, i.e. comparing age class "older than 6 years" with age class "1–3 years" an Odds ratio of 4.0 (CI: 2.75; 5.67 $p < 0.001$) was calculated. In bulls older than 3 years and mainly used as service bulls, prevalences of 14.3 % were observed. In bulls not yet introduced to service and less than 3 years of age, 3.5 % were recorded. This corresponds to an Odds ratio of 4.6 (CI: 1.25; 13.84; $p < 0.05$) for the exposure factor "service bull >3 years" and seropositivity.

In Guinea Bissau 30.6 % percent (49/160) of CFT positive cattle were older than 6 years, 16.6 % (40/241) 3 – 6 years and 6.9 % (21/305) 1 – 3 years. In calves 3.7 % reacted positive (1/27). From the age related differences in prevalences, i.e. comparing age class "older than 6 years" with age class "1– 3 years" an Odds ratio of 8.1 (CI: 4.51, 14.75; $p < 0.05$) was calculated. In bulls older than 3 years and mainly used as service bulls, prevalences of 23.8 % were observed. In bulls not yet introduced to service and less than 3 years of age 4.4% were recorded. This corresponds to an Odds ratio of 6.8 (CI: 1.37, 32.60; $p < 0.01$) for the exposure factor "service bull > 3 years" and seropositivity.

Senegal and The Gambia

The few brucellosis positive cattle found in Senegal (n= 3) and The Gambia (n= 5) were with one exception (8 months old calf) between 5 and 9 years old.

Table 5: Relationships of individual animal data and seropositivity for Guinea (4 Districts) and Guinea Bissau (2 districts)

	Guinea Bissau				**Guinea** (Labé* not included)			
	No. cattle	Propor-tion (%)	No CFT positive	No CFT positive (%)	No. cattle	Propor-tion (%)	No CFT positive	No CFT positive (%)
Category of animals								
Total	**733**	**100**	**111**	**15.1**	**3051**	**100**	**275**	**9.0**
Heifers	211	28.8	18	8.5 [1]	1064	34.9	54	5.1[1]
Cows	360	49.1	82	22.8 [2]	1027	33.7	128	12.5 [2]
Calves (0.5 - <1year)	27	3.7	1	3.7 [1]	383	12.6	8	2.1[1]
Bulls (1-3 years)	114	15.6	5	4.4 [1]	542	17.8	19	3.5[1]
Bulls (> 3 years)	21	2.9	5	23.8 [2]	35	1.1	5	14.3 [2]
Age								
(0.5 -) < 1 year	27	3.7	1	3.7[a]	383	12.6	8	2,1 [a]
1 - 3 years	305	41.6	21	6.9 [a]	1527	50.2	62	4,1 [a]
>3 - 6 years	241	32.9	40	16.6 [b]	575	18.9	63	11,0 [b]
> 6 years	160	21.8	49	30.6 [c]	566	18.4	81	14,3 [b]

* Labé was excluded because of absence of the disease
Superscript letters and figures: Significant differences between categories ($p < 0.05$)

3.5 Individual animal prevalence and clinical observations

Odds ratio was calculated for likely associations between the brucellosis status of individuals and the factors "observation of hygroma" (Tab 6) and "occurrence of abortions" during the last 12 months (Tab 7).

Clinical hygroma observed and seropositivity

127 cows with hygroma were reported in Guinea and 59 in Guinea Bissau during the study period. Odds ratios of 209.3 (120.27, 377.73; p< 0.001) for Guinea and 65.2 (CI: 28.66, 164.73; p< 0.001) for Guinea Bissau for the factor "observed hygroma" at the farm visit indicate, that this factor is highly related to seropositivity.

In three of the five identified serologically positive cattle reported for The Gambia (CRD) clinical hygromas were observed. In Senegal (Bassin Arachidier) three serologically positive cattle were identified, one animal with hygroma was found.

Table 6: Seropositivity in individual cattle and clinical sign of hygromas

	Cattle with observed hygroma at farm visit		
	Clinical **hygromas** in **sero +*ve* cattle** in **%**	Clinical **hygromas** in **sero -*ve* cattle** in **%**	OR*
Overall	**49.2 (164/333)**	**0.5 (26/5205)**	**193.3** (121.95, 308.34)
Guinea	50.9 (109/214)	0.5 (18/3647)	209.3 (120.27, 377.73)
Guinea Bissau	45.9 (51/111)	1.3 (8/622)	65.2 (28.66, 164.73)
The Gambia	60.0 (3/5)	(0/460)	-
Senegal	33.3 (1/3)	(0/476)	-

* Exposure factor: seropositivity and the presence of hygroma

Abortion history in heifers and cows and seropositivity (table 7)

With an abortion occurrence of 18.1 % (33/182) in Guinea and 31.0 % (31/100) for Guinea Bissau seropositive animals were eight respectively ten times higher at risk to

have a previous abortion observed than seronegative animals (OR for Guinea: 8.0, CI: 4.91, 12.68; p< 0.001; OR for Guinea Bissau: 9.6, CI: 5.01, 18.61; p< 0.001).
In all female cattle found seropositive in The Gambia (CRD) and Senegal (Bassin Arachidier) no abortions occurred during the last 12 months.

The average of abortion rate in seronegative cattle was 2.6 % with a range from 0.9 to 4.5 between the countries.

Table 7: Seropositivity in individual cattle (heifers and cows) and abortion history

	Observed abortion during last 12 months		
	Sero +ve cattle* with abortion history in %	Sero -ve cattle* with abortion history in %	OR**
Overall	**22.1 (64/290)**	**2.6 (96/3636)**	**10.4 (7.27, 14.90)**
Guinea	18.1 (33/182)	2.7 (67/2476)	8.0 (4.91,1.68)
Guinea Bissau	31.0 (31/100)	4.5 (21/471)	9.6 (5.01, 18.61)
The Gambia	(0/5)	0.9 (3/319)	-
Senegal	(0/3)	1.3 (5/370)	-

* Heifers and cows
** Exposure factor: seropositivity and occurrence of abortions (last 12 months)

3.6 Within-herd prevalence rates

With the exception of the district of Labé (Guinea) where non positive reactor was found, the *within-herd prevalence* rates showed a wide variability within the districts in the 4 countries (Annex 1-4).

In Guinea the *within-herd prevalence* rates ranged in Dubréka from 0 to 38% with a mean of 13.2%, in Boké from 0 to 31% (mean of 6.6%), in Coyah from 0 to 18 % (mean of 5.9 %) and in Forehcariah from 0 to 16 % with a mean of 3.5 % (Annex: 1). In Guinea Bissau the *within-herd prevalences* varied in Bafata from 3.0 % to 48 % with a mean of 19.4 % and in Gabu from 0 to 15.0 % with a mean of 5.7 % respectively (Annex 2). At the three positive farms in Senegal (Annex 3) and in The Gambia (Annex 4) *within-herd prevalences* ranged from 4 % to 17 % and 2 % to 11 % respectively.

High *within-herd prevalences* (≥ 10 %) were mainly reported for herds in Guinea and Guinea Bissau (Table 8). In Guinea ten out of 19 farms in Dubréka, five out of 18 farms in Coyah, four out of 17 farms in Boké and two out of 18 farms in Forehcariah fall into this category. In Bafata ten out of 15 farms were classified accordingly and in Gabu two out of 9. In Senegal (Bassin Arachidier) and The Gambia (CRD south) only on one farm such high prevalences were reported in each country.

Negative herds were predominant in the sampled districts of Senegal (27/30), The Gambia (17/20) and Labe district of Guinea, while in all other study areas in Guinea and Guinea Bissau brucellosis negative herds were less frequent than positive herds (Tab 8).

Table 8: Frequency of high prevalence (>10%) and sero-negative herds

Region Area	Herds sampled	*Within-herd prevalence rate* **> 10% (no. of herds)**	**Herds –*ve* (no. of herds)**
Guinea			
Labé	18	0	18
Boké	19	4	5
Dubréka	17	10	1
Coyah	18	5	3
Forehcariah	18	2	7
Guinea Bissau			
Bafata	15	10	0
Gabu	9	2	2
The Gambia			
CRD south	20	1	17
Senegal			
Bassin Arachidier	30	1	27

The *overall prevalence rate in bulk milk* samples based on the results of the milk ELISA applied on some farms in Dubréka and Boké (Guinea) and Bafata (Guinea Bissau) was 85.7 % (30/35). 80% of the herds in Dubréka (8/10), 75% of the herds in Boké (9/12) and 87% (13/15) of the herds in Bafata were considered positive. The serological screening of individual sera revealed that all milk ELISA-positive herds had at least one CFT reactor in the corresponding herd. Four serologically positive herds were negative in the milk ELISA (Annex 1 and 2).

In The Gambia and Senegal, positive bulk milk samples were always found on serologically positive farms, at a prevalence rate of 15 % and 10 % respectively (Annex 3 and 4).

3.7 Within-herd prevalence and herd size

Based on results obtained from the questionnaire a likely association between herd size and herd brucellosis status was found (Tab 9). The herd sizes were categorized according to quartiles, 1st for small, 2nd & 3rd medium and 4th quartile for large herds. Results indicate an increase of the *within-herd prevalence* with greater herd size, which is significant between small and large herds ($p < 0.05$) for Guinea and Guinea Bissau. Moreover, the proportion of serologically negative herds decreases with increasing herd size in both countries, with significant differences between small and medium versus large herds ($p < 0.05$) for Guinea.

Table 9: Relationship of herd size and brucellosis herd status for Guinea and Guinea Bissau

Guinea (Labé excluded)*		**Guinea Bissau**	
Herd status (n= 72)	Within-herd prevalence (CFT -*ve* herds)	Herd status (n= 20)	Within-herd prevalence (CFT -*ve* herds)
Small size	**7.0 %**[a]	**Small size**	**5.9 %** [a]
1st quartile (<69 cattle)	n= 18	1st quartile (<28 cattle)	n= 6
–ve herds	(8/18) [a]	–ve herds	(2/6)
Medium size	**9.2%**[a]	**Medium size**	**12.9 %** [a]
2nd & 3rd quartile (70-126 cattle)	n= 36	2nd & 3rd quartile (28-171 cattle)	n= 12
–ve herds	(1/36) [a]	–ve herds	(0/10)
Large size	**11.1%**[b]	**Large size**	**24.4 %** [b]
4th quartile (>126 cattle)	n= 18	4th quartile (>171 cattle)	n= 6
–ve herds	(1/18) [b]	–ve herds	(0/5)

* Labé was excluded because of absence of the disease
Superscript letters: Significant differences between categories ($p < 0.05$)

3.8 Knowledge of farmers on brucellosis

When interviewed on knowledge of brucellosis and its zoonotic character, some differences in responses between the districts respectively countries were observed. In general it appears, that knowledge of farmers in Guinea Bissau is better than elsewhere.

With the exception of the Bassin Arachidier (Senegal) and Labé (Guinea) the majority of farmers in all other districts have at least some knowledge of brucellosis, i.e. hygroma as one of the symptoms. Milk from cows with hygromas or previous abortion history was consumed or sold at the local market by all farms in Guinea, The Gambia and Senegal, but some farmers in Guinea Bissau discard milk form these animals. However, such cattle are never separated from the herd in any farm of the 4 countries.

Only a small proportion of selected farmers in Guinea (8/90), The Gambia (2/20) and Senegal (4/30) had some knowledge on the zoonotic character of the disease. The knowledge seems to be again higher in farmers in Guinea Bissau (6/31).

Heat treatment of milk is usually not applied in Guinea (1/90), Senegal (1/30) and The Gambia (0/20). The majority of farmers in Guinea Bissau (19/31) however, is heat treating their milk.

On seven farms in Guinea (Dubréka: TDN 4-6, 7, 14; Boké: KBE 3, Coyah: Herd ID 9) and on three in Guinea Bissau (Bafata: ID 12, 17; Gabu: ID 7, 12, 13) people reported trustfully that they had suffered from brucellosis-like symptoms previously. On all of these farms positive cattle were reported. From five of these farms (TDE 6, 7, 14; Bafata 12, 17) bulk milk samples were collected and found positive.

Table 8: Knowledge of farmers on brucellosis and the zoonotic character

	Knowledge on symptoms in cattle	Milk discharged from cows with hygroma	Milk treatment applied	Knowledge on zoonotic character	Reports on suspected Bruc. in man
Guinea					
Labé	6% (1/18)	(0/18)	(0/18)	(0/18)	(0/18)
Boké	89% (17/19)	(0/19)	(0/19)	5% (1/19)	5% (1/19)
Dubréka	100% (17/17)	(0/17)	6% (1/17)	3% (5/17)	29% (5/17)
Coyah	83% (15/18)	(0/18)	(0/18)	1% (2/18)	6% (1/18)
Forehcariah	100% (18/18)	(0/18)	(0/18)	(0/18)	(0/18)
Guinea Bissau					
Bafata	100% (15/15)	33% (5/15)	60% (9/15)	27% (4/15)	13% (2/15)
Gabu	100% (9/9)	22% (2/9)	33% (3/9)	11% (1/9)	33% (3/9)
The Gambia					
CRD south	60% (12/20)	(0/20)	(0/20)	10% (2/20)	(0/20)
Senegal					
Bassin Arachidier	17% (5/30)	(0/30)	3% (1/30)	13% (4/30)	(0/30)

3.9 Seroprevalences in man living in a high prevalence area for brucellosis in cattle

In Dubréka District of Guinea, a high prevalence area for cattle in this study, some members of potential risk groups (8 herdsmen, 7 herd owners, 3 milk vendors and 2 veterinary technicians) volunteered to be tested for the presence of *Brucella abortus* antibodies. Out of 20 volunteers seven reacted positive, all of them belonged to the groups of herders (n= 4) or herd owners (n= 3).

4 DISCUSSION

All serum samples examined for the estimation of brucellosis prevalence were subjected to the RBT as screening and the CFT as confirmatory test, as recommended as standard procedure by OIE (2001). A high degree of agreement between both tests is normally expected (HADAD and JAMALLUDEEN, 1992; STAAK, 1990). In our study, however, 26% of the positive samples in RBT could not be confirmed in CFT. Given the different level of laboratory standards in the four countries where the RBT was carried out, a possible explanation for this disagreement between RBT and CFT could be that the RBT antigen became contaminated or expired. Antigen and sera may not have been brought up to room temperature before testing. Also, an overestimation of the agglutination reaction by the individual investigator could be considered. Moreover, cross-reactions with other bacteria could have led to false positive reactions (BLOBEL and SCHLIESSER, 1982). However, according to NAKAVUMA (1994) the RBT provides more likely false positive results than to miss brucellosis. Hence, the confirmatory test CFT, which was carried out only at ITC and DNE laboratories, reduced the number of positive samples considerably.

In principle all titres can be attributed to natural infections since residual vaccination titres i.e. due to S 19 vaccination as well as titres from ingestion of colostrum of infective dams can be excluded; no vaccinations were carried out in the past and all cattle were older than 6 months.

Results indicate different epidemiological situations for brucellosis in the four countries. Highest average mean prevalences of all animals were found in Guinea Bissau with 15.1 %, followed by Guinea with 7.2 %. Considerable lower numbers of cattle reacted in Senegal and The Gambia with 0.6. % and 1.1 % respectively. In addition, the epidemiological situation differed clearly between districts within the countries. As previous reports are rare, obsolete and usually not representative it is difficult to use them for comparison with own results. However, in the absence of more reliable observations they will be used for discussion of results.

An average individual prevalence for brucellosis for Basse Guinea based on literature available and data from a countrywide survey in 1997 (CONDE, 2000) of 7.5 % was assumed with wide variations between the districts (0 – 20%).

While individual prevalences reported for Boké District in 1997 (6.9 %) were confirmed by results of our study, herd prevalences in this study (74%) clearly differ from 1997 results (48%). For Dubréka we found higher prevalences at herd and individual level in the current study than in the survey of 1997. For Coyah and Forehcariah higher individual prevalences (20% and 16,7%) where observed in 1997, whereas the herd prevalences did not differ much (71% and 70%) from these observations. However, sampling in 1997 was not representative for the district cattle populations, e.g. in Coyah, Forehcariah and Dubréka only an average of 3.9 – 6.0 cattle per farm were sampled, therefore it is assumed that results of the 1997 survey may not represent the true epidemiological situation.

The significantly higher individual animal and within-herd prevalences for Dubréka district may be attributed to the common practice of transhumance in this district. Other management and husbandry factors did not differ much between the five study districts. Most of the herds in Dubréka are in transhumance in March, and moved to communal pasture (“parc”) where they remain until June. The communal pastures are also used by herds from Fria and Boffa Districts. All herds, including bulls, are usually not separated. In particular, at the commonly used communal water points animals from different locations and likely different status for various infections (including brucellosis) are in close contact.

In contrast to the moderate to high prevalences in Coyah, Forehcariah, Dubréka and Boké there was an absence of brucellosis infections in Labé. This result was not expected as 25% positive responders in RBT were reported in October 2000 when 120 cattle from farms located in Labé and selected for artificial insemination were tested. Based on our observations all samples from 2000 were retested using parallel testing

(RBT and CFT) with a new batch of antigen and they were found negative, hence confirming negative results of this study for Labé (Goumou, 2002).

In Bafata, Guinea Bissau, brucellosis prevalence rates according to DGP (2000) and oral reports are expected to be in the range of 10 to 40 % and were confirmed in our study (18.6%). For Gabu no recent information on brucellosis prevalence confirmed by an acknowledged test (i.e. MRT, RBT, ELISA, CFT) was available but oral reports on frequently observed hygromas and abortions in cattle are in agreement with our serological findings for individuals (5.7 %).

The very low prevalence reported for The Gambia is in agreement with previous ITC observations made between 1995 and 1999 when 131 cattle with previous abortion history where tested for brucellosis (Unger, 2002). As only 2% reacted positive in the RBT it was assumed that the observed abortions were related to other infections than to brucellosis.

For the Bassin Arachidier in Senegal KONTE (1994) reported average prevalence rates of 8.9 %. More recent NDOUR (2003) stated prevalences between 2 and 4.6 % for Sindia-Nguekokh, a sub region within the Bassin Arachidier. Prevalences reported in this survey (0.6 %) were considerably lower and might be explained by differences in the study locations within the Bassin Arachidier.

In agreement with other authors (AKAKPO and BORNAREL, 1987; OLOFFS 1996, 1998), individual seroprevalences in this study increased with age. Cattle older than 6 years were 3.5 times higher at risk to be seropositive for brucellosis than 1-3 years old animals. As 90% of diseased animals are infected live-long, the chance to become infected increases with age. Low prevalences established for calves support a reduced risk of infection at early age.

According to the "epizootical rule" (AKAKPO and BORNAREL, 1987) "small herds - low incidence, large herd - high incidence", the within-herd prevalences were related significantly to herd size. Large herds had a higher average within-herd prevalence than small herds. Moreover, negative herds were more likely to be found among small herds than in large herds. Similar results were found by OLOFFS (1996) in Uganda.

Frequencies of hygroma and abortions in individual animals were significantly related to seropositivity, as also reported by AKAKPO and BORNAREL (1987) and OLOFFS et al. (1998). A small proportion of seronegative cattle had also aborted during the 12 months preceding the study, indicating that infections other than brucellosis may cause abortions.

Positive results for bulk milk samples using MRT on farms in The Gambia and Senegal or milk-ELISA applied on some farms in Guinea and Guinea Bissau coincided 100% with positive serological results at herd level. However, despite the high sensitivity of the ELISA test some herds in Guinea and Guinea Bissau (within-herd prevalence generally less than 8%) reacted negative. Possible explanations are: Firstly, not all serologically positive lactating cows were sampled during this herd screening. Secondly, the phenomenon of intermittent shedding of antibodies via milk. These results agree with findings of other investigations. BLOBEL and SCHLIESSER (1982) therefore recommend that one–time screening of bulk milk samples is insufficient to identify brucellosis-affected herds.

The knowledge of farmers on the zoonotic character and the ways of transmission of the disease in cattle was generally poor but differs between the countries. Symptoms of brucellosis in cattle and the possible ways of transmission to man, were better known in the high infection areas of Guinea Bissau and Guinea. However, this difference in knowledge did not result in the application of any meaningful control measures, thus may not contribute to reduced prevalences in cattle.

The reported observations on suspected brucellosis-like symptoms in man on serologically positive farms, the positive bulk milk samples as well as the serological positive tested volunteers in Dubréka in Guinea indicate the associated high public health risk.

5 CONCLUSIONS

Brucellosis herd prevalences, determined by means of serology and milk testing and the reported suspected (Guinea Bissau and Guinea) and confirmed cases of brucellosis in humans in Guinea (Dubréka) indicate a public health risk for consumers of milk, particularly where the milk is consumed untreated like in the study area. As the knowledge of farmers on the transmission of the disease in cattle and on its zoonotic aspect is poor, there is an urgent need to improve knowledge about the disease. Risk groups (farmers, milk sellers and consumers) should be informed and made aware of the character of the disease, the related public health risk and how to avoid infection. In particular, public health authorities have to be mobilized in a similar manner as demanded for other high risk areas in Kenya and Uganda (McDERMOTT and ARIMI, 2002) where also little awareness about the importance of brucellosis in man was found.

In the study regions the traditional farming system is predominant. However, first steps towards intensification are already undertaken, i.e. through introduction of crossbred cattle in the peri-urban areas of Guinea. Brucellosis is considered an infection, which increases with the degree of intensification in animal production (BLANKENBURG and CREMER, 1999). As prevalences are already high in the traditional system, the process of intensification might result in an even further increased public health risk.

According to recommendations of the “Task Force, Subgroup Bovine Brucellosis” (EU, 2000) the introduction of vaccination of heifer calves in herds with confirmed prevalences over 10% (reported for 4 districts of Guinea and all in Guinea Bissau) is recommended in order to reduce the incidence to levels at which a subsequent eradication programme, based on a test and slaughter policy, can be initiated. In infected herds with less than 10 % prevalence (CRD in The Gambia, Bassin Arachidier in Senegal and Labé in Guinea) culling of positive reactors is the preferred control option. Non-infected herds should be retested annually.

Livestock offers both a major contribution to the livelihood of producers but also a risk to their and to consumers´ health and performance. The control of zoonotic diseases like brucellosis will require a coordinated effort of medical and veterinary research, the provision of veterinary services and the reinforcement of disease control regulations.

6 REFERENCES

Akakpo, A. J., Bornarel, P.(1987):
Epidemiology of animal brucellosis in Tropical Africa:
Clinical, serological, and bacteriological surveys.
Res. Sci. Tech. 6, 981-1027.

Anon., (1986):
Joint FAO/WHO Expert Committee on Brucellosis.
World Health Organization, Technical Report Series, WHO Geneva, 740 pp.

Von Blankenburg, P., Cremer, H.D. (1999):
Handbuch der Landwirtschaft und Ernährung in den Entwicklungsländern.
BD. 5.: Tierzucht in den Tropen und Subtropen.
Ulmer Verlag, Stuttgard, 96 – 113.

Blobel, H., Schliesser,T. (1982):
Handbuch der bakteriellen Infektionen bei Tieren, Band IV.
Jena: VEB Gustav Fischer Verlag, 53-213 and 408-465.

Conde M.C. (2000)
Situation de la Brucellose en Guinee
Premieres Journees Nationales Veterinaires.
DNE, Conakry, Guinee, 7-8 November 2000

Djordjevic, M., Lako, B., Ristanovic, E. (2003):
The significance of serological tests in diagnosis and following up of neurobrucellosis:
case report. Poster.
Brucellosis 2003 International Research Conference.
Sep 15-17, 2003, University of Navarra, Pamplona, Spain.

DGP (2000):
Personal communication.
Direcao Geral Percuria, Guinea Bissau.

EU (2000):
European Commission, Health and Consumer Protection Directorate General
Unit 52 - Legislation on animal health and live animals
Members of the task force, Subgroup Bovine Brucellosis
Bruxelles/Wetstraat, Belgium, 2000.

Goumou, A. (2002):
Personal communication.
DNE, Conakry.

Hadad, J.J., Jamalludeen, N.M.A., (1992):
The prevalence of brucellosis in cattle in Ninevah province, Iraq.
Iraqi Journal of Veterinary Sciences 5, 159-164.

Hahn, H. (1991):
Medizinische Mikrobiologie.
Berlin: Springer Verlag, 372-375.

Konte, M. (1994):
Pathologie de la reproduction chez les bovins du Sénégal. Séro-épidémiologie des maladies bactériennes. Mise au point d'une sonde de détection des leptospires pathogènes par la technique PCR.
Th. de doct. Sc., ACAD Dakar, N°39, 1994, 216p.

Masouridou, S., Andriopoulos, P., Fokas, S., Kalkani, M., Dinosopoulou, M., Asimakopoulos, G.. Tsironi, M. (2003):
Presentation of acute brucellosis: A review of 144 casis.
Brucellosis 2003 International Research Conference.
Sep 15-17, 2003, University of Navarra, Pamplona, Spain.

Nakavuma, J. (1994):
A serological survey of *Brucella abortus* in cattle and goats in the central and southern regions of Uganda.
M.Sc. Thesis, Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda.

Ndour A. E. (2003):
Dynamique du statute sanitaire et des performances de production des vaches laitières dans le bassin arachidier du Sénégal: cas de la zone de Sindia-Nguekokh.
Th. doct. vét., Dakar, 2003, 194p.

Nicoletti,P. (1984):
Control of Brucellosis in tropical and subtropical regions.
Prev. Vet. Med. 2, 193-196.

Mc Dermott, J.J., Arimi, S.M. (2002)
Brucellosis in sub-Saharan Africa: epidemiology, control and impact.
Vet. Microbiol. 90, 111 – 134.

O.I.E. (Office International des Èpizooties), (1987):
Brucellosis in cattle, sheep and goats.
Technical series No. 6., 9-35.

O.I.E. (Office International des Èpizopoties), (2001):
World Organisation for Animal Health
Bovine Brucellosis, Manual of Standards, Diagnostic test and Vaccines
Part 2, Section 2,3, Chapter 2.3.1., Paris.

Oloffs A. 1996.
Bovine brucellosis investigations on selected dairy farms in Rukungiri District and Mukono Country, Uganda.
Diploma Thesis, Freie Universität Berlin, Fachbereich Veterinärmedizin, Berlin, Germany

Oloffs A., Baumann M.P.O., Afema J., Nakavuma J. (1998)
Experiences with a strategy to investigate bovine brucellosis in a rural area in Southwest Uganda.
Revue Èlev. Méd. Vét. Pays trop., 51 (2), 101-105

Perry, B.D., Randolph J.F., McDermott, J.J., Sones, K.R., Thornton, P.K. (2002)
Investing in animal health research to alleviate poverty
ILRI, Nairobi, 2002

Staak, C. (1990):
Serological techniques in brucellosis and interpretation of results.
Presentation at the 1st International Conference on Brucellosis 19/20. March, 1990, Mosul, Iraq (unpublished).

Thrusfield, M. (1995):
Veterinary Epidemiology, 2nd edition.
Oxford: Blackwell Science, 280-282.

Unger, F. (2002):
Brucellosis in cattle from selected districts in The Gambia with previous abortion history.
ITC, 2002, (unpublished)

7 ANNEX

Annex 1-4: Brucellosis within-herd prevalence rates established by CFT for all districts sampled.

Annex 1: Brucellosis within-herd prevalence rates in Guinea (5 Districts)

District	Farm ID	Herd size	No. tested	No. CFT positive	Within-herd prevalence CFT positive animals (%)	Milk ELISA results
DUB	TDE 7	68	26	10	38.5	+ ve
DUB	TDE 20	150	45	11	24.4	+ ve
DUB	TDE 18	96	45	10	22.2	+ ve
DUB	TDE 17	198	50	10	20.0	+ ve
DUB	TDN 1	62	45	9	20.0	*
DUB	TDE 5	96	45	7	15.6	*
DUB	TDN 4	73	45	6	13.3	*
DUB	TDN 6	130	45	6	13.3	*
DUB	TDE 16	133	45	5	11.1	+ ve
DUB	TDN 5	58	45	5	11.1	*
DUB	TDE 6	80	48	5	10.4	+ ve
DUB	TDE 11	80	40	3	7.5	+ ve
DUB	TDN 3	78	45	3	6.7	*
DUB	TDE 14	55	55	3	5.5	+ ve
DUB	TDE 4	73	35	1	2.9	- ve
DUB	TDE 15	96	45	1	2.2	- ve
DUB	TDN 2	59	45	0	0	*
DUB	**Total**	**1585**	**749**	**95**	**13.2 (10.8,15.6)** [a]	
BOK	KBE34	102	26	8	30.8	+ ve
BOK	CBE34	56	25	4	16.0	+ ve
BOK	CBE 36	113	50	7	14.0	+ ve
BOK	CBE 35	127	45	6	13.3	+ ve
BOK	KBE 31	75	25	2	8.0	*
BOK	KBE 24	49	27	2	7.4	- ve
BOK	CBE9	78	47	3	6.4	+ ve
BOK	KBE 10	55	32	2	6.3	*
BOK	KBE22	70	32	2	6.3	+ ve
BOK	KBE20	150	45	2	4.4	- ve
BOK	KBE29	57	25	1	4.0	+ ve
BOK	KBE 23	140	26	1	3.8	+ ve
BOK	KBE 30	121	40	1	2.5	+ ve
BOK	KBE 38	110	50	1	2.0	*
BOK	KBE 14	49	25	0	0	- ve
BOK	KBE 15	47	32	0	0	*
BOK	KBE 21	58	33	0	0	*
BOK	KBE 36	65	40	0	0	*
BOK	KBE37	50	50	0	0	*
BOK	**Total**	**1572**	**675**	**42**	**6.6 (4.7,8.5)** [b]	

Coyah	5	130	45	8	17.8	*
Coyah	9	83	45	7	15.6	*
Coyah	3	80	45	6	13.3	*
Coyah	10	68	45	5	11.1	*
Coyah	18	57	45	5	11.1	*
Coyah	14	59	45	3	6.7	*
Coyah	2	89	45	2	4.4	*
Coyah	4	139	45	2	4.4	*
Coyah	6	167	45	2	4.4	*
Coyah	8	140	45	2	4.4	*
Coyah	11	75	45	2	4.4	*
Coyah	1	90	45	1	2.2	*
Coyah	7	116	45	1	2.2	*
Coyah	12	98	45	1	2.2	*
Coyah	15	215	45	1	2.2	*
Coyah	13	150	45	0	0	*
Coyah	16	58	45	0	0	*
Coyah	17	65	45	0	0	*
Coyah	**Total**	**1879**	**810**	**48**	**5.9 (4.3,7.5)** [b]	
Forehcariah	6	132	25**	4	16.0	*
Forehcariah	18	132	52	7	13.5	*
Forehcariah	2	170	45	4	8.9	*
Forehcariah	3	85	45	4	8.9	*
Forehcariah	16	96	45	3	6.7	*
Forehcariah	1	130	45	2	4.4	*
Forehcariah	4	98	45	1	2.2	*
Forehcariah	7	89	45	1	2.2	*
Forehcariah	9	103	45	1	2.2	*
Forehcariah	14	123	45	1	2.2	*
Forehcariah	12	160	65	1	1.5	*
Forehcariah	5	124	45	0	0	*
Forehcariah	8	122	45	0	0	*
Forehcariah	10	124	45	0	0	*
Forehcariah	11	109	45	0	0	*
Forehcariah	13	78	45	0	0	*
Forehcariah	15	190	45	0	0	*
Forehcariah	17	124	45	0	0	*
Forehcariah Total		**2189**	**817**	**29**	**3.5 (2.2,4.8)** [b]	
Labé	1	60	45	0	0	*
Labé	2	80	45	0	0	*
Labé	3	55	45	0	0	*
Labé	4	100	45	0	0	*
Labé	5	120	45	0	0	*
Labé	6	60	45	0	0	*
Labé	7	50	45	0	0	*
Labé	8	75	45	0	0	*
Labé	9	85	45	0	0	*

Labé	10	68	45	0	0	*
Labé	11	78	45	0	0	*
Labé	12	90	45	0	0	*
Labé	13	70	45	0	0	*
Labé	14	80	45	0	0	*
Labé	15	74	45	0	0	*
Labé	16	52	45	0	0	*
Labé	17	60	45	0	0	*
Labé	18	65	45	0	0	*
Labé	**Total**	**1322**	**810**	**0**	**0**	*

* Not tested!   a:b ($p < 0.05$)

** In 5.6% (1/18) farms sampled in Forehcariah the correct sample size was lower by 1 animals, which escaped in the morning of sampling.

Annex 2: Brucellosis within-herd prevalence rates in Guinea Bissau (2 Districts)

District	Farm ID	Herd size	No. tested	No. CFT positive	Within-herd prevalence CFT positive animals (%)	Milk ELISA results
Bafata	11	353	25**	12	48.0	+ ve
Bafata	9	135	30	11	36.7	+ ve
Bafata	14	94	30	9	30.0	+ ve
Bafata	10	39	25	7	28.0	+ ve
Bafata	2	285	45	12	26.7	+ ve
Bafata	6	172	45	10	22.2	+ ve
Bafata	17	167	41	9	22.0	+ ve
Bafata	3	450	45	8	17.8	+ ve
Bafata	16	156	45	6	13.3	+ ve
Bafata	4	304	37	4	10.8	+ ve
Bafata	12	113	40	4	10.0	+ ve
Bafata	1	25	21	2	9.5	+ ve
Bafata	5	270	45	3	6.7	- ve
Bafata	7	87	32	2	6.3	+ ve
Bafata	8	171	33	1	3.0	- ve
Bafata	**Total**	**2821**	**539**	**100**	**19.4 (16.1,22.7)** [a]	
Gabu	12	26	20	3	15.0	*
Gabu	5	16	14	2	14.3	*
Gabu	8	86	25	2	8.0	*
Gabu	6	36	32	1	3.1	*
Gabu	11	40	34	1	2.9	*
Gabu	13	19	16	1	6.3	*
Gabu	14	20	18	1	5.6	*
Gabu	1	20	18	0	0	*
Gabu	7	20	17	0	0	*
Gabu	**Total**	**283**	**194**	**11**	**5.7 (1.8,9.6)** [b]	

* Not tested!   a:b ($p < 0.05$)

** In 7% (1/15) farms sampled in Bafata the correct sample size was lower by 3 animals, which escaped in the morning of sampling.

Annex 3: Brucellosis within-herd prevalence rates in Senegal (1 region)

District	Farm ID	Herd size	No. tested	No. CFT positive	Within-herd prevalence CFT positive animals (in %)	Milk ELISA results
Bassin Arachidier	Z16	7	6	1	16.7	+ve
Bassin Arachidier	Z3	19	17	1	5.9	+ve
Bassin Arachidier	Z12	27	23	1	4.3	+ve
Bassin Arachidier	Z2	6	4	0	0	-ve
Bassin Arachidier	Z5	20	18	0	0	-ve
Bassin Arachidier	Z6	9	7	0	0	-ve
Bassin Arachidier	Z7	15	12	0	0	-ve
Bassin Arachidier	Z8	12	8	0	0	-ve
Bassin Arachidier	Z9	5	4	0	0	-ve
Bassin Arachidier	Z13	20	20	0	0	-ve
Bassin Arachidier	Z14	25	24	0	0	-ve
Bassin Arachidier	Z15	32	28	0	0	-ve
Bassin Arachidier	Z19	27	24	0	0	-ve
Bassin Arachidier	Z21	20	18	0	0	-ve
Bassin Arachidier	Z22	15	15	0	0	-ve
Bassin Arachidier	Z24	10	8	0	0	-ve
Bassin Arachidier	Z25	32	28	0	0	-ve
Bassin Arachidier	Z27	33	28	0	0	-ve
Bassin Arachidier	Z28	20	17	0	0	-ve
Bassin Arachidier	Z29	32	28	0	0	-ve
Bassin Arachidier	Z30	12	10	0	0	-ve
Bassin Arachidier	m2	18	15	0	0	-ve
Bassin Arachidier	m3	12	10	0	0	-ve
Bassin Arachidier	m4	20	18	0	0	-ve
Bassin Arachidier	m5	13	13	0	0	-ve
Bassin Arachidier	m6	16	14	0	0	-ve
Bassin Arachidier	m15	1	1	0	0	-ve
Bassin Arachidier	m10	26	23	0	0	-ve
Bassin Arachidier	m13	4	3	0	0	-ve
Bassin Arachidier	m16	39	35	0	0	-ve
Bassin Arachidier Total		**547**	**479**	**3**	**0.9 (0,1.1)**	

* Not tested!   a:b ($p < 0.05$)

Annex 4: Brucellosis within-herd prevalence rates in The Gambia (1 District)

District	Farm ID	Herd size	No tested	No. CFT positive	Within-herd prevalence CFT positive animals (in %)	Milk ELISA results
CRD	11	23	19	2	10.5	+ve
CRD	17	30	28	1	3.6	+ve
CRD	20	100	87	2	2.3	+ve
CRD	1	18	16	0	0	-ve
CRD	2	16	12	0	0	-ve
CRD	3	25	21	0	0	-ve
CRD	4	31	28	0	0	-ve
CRD	5	11	11	0	0	-ve
CRD	6	27	24	0	0	-ve
CRD	7	14	12	0	0	-ve
CRD	8	20	16	0	0	-ve
CRD	9	16	15	0	0	-ve
CRD	10	32	28	0	0	-ve
CRD	12	30	27	0	0	-ve
CRD	13	9	9	0	0	-ve
CRD	14	10	7	0	0	-ve
CRD	15	35	32	0	0	-ve
CRD	16	50	47	0	0	-ve
CRD	18	12	12	0	0	-ve
CRD	19	15	14	0	0	-ve
CRD	**Total**	**524**	**465**	**5**	**0.8 (0,2.0)**	

* Not tested! a:b ($p < 0.05$)