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THE OCCURRENCE OF INFECTIOUS DISEASES IN MIXED FARMING OF DOMESTICATED WILD HERBIVORES AND LIVESTOCK IN KENYA. II. BACTERIAL DISEASES[1]

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ABSTRACT: The prevalence of antibodies to *Brucella* spp., *Mycobacterium paratuberculosis* and the *Mycoplasma* spp. causing contagious bovine pleuropneumonia and contagious caprine pleuropneumonia was determined in various species of ruminants on a ranch in the semi-arid zone of southeastern Kenya. Antibody titers to *Brucella* spp. were found in eland (*Taurotragus oryx*), oryx (*Oryx beisa*) and camels (*Camelus dromedarius*). Reactors were not found in buffalo (*Syncerus caffer*), sheep (*Ovis aries*) and goats (*Capra hircus*). *Brucella* sp. was not isolated from eland and camels. Antibody titers to *M. paratuberculosis* were found only in camels and goats. Mycobacteria were not detected in feces of two serologically positive camels. Significant serum antibody titers to *Mycoplasma mycoides mycoides* were found only in camels. Antibody titers to *Mycoplasma* sp. (strain F38), which causes contagious caprine pleuropneumonia, were found in buffalo, cattle and camels but not in the other species. Attempts to isolate the *Mycoplasma* sp. from nasal secretion of the buffalo and camels failed. The possible occurrence of tuberculosis in camels is discussed. Under the conditions at the ranch, contagious bacterial diseases appear to be of minor importance in the domesticated wild herbivores. The introduced camels, however, might be a source of various infections such as brucellosis, mycoplasmosis and possibly tuberculosis for the other susceptible species.

Key words: Infectious diseases, bacteria, mycoplasmosis, brucellosis, tuberculosis, livestock, camels, wildlife, Africa.

INTRODUCTION

Domestication of oryx (*Oryx beisa*), eland (*Taurotragus oryx*) and buffalo (*Syncerus caffer*) was started in 1970 on Galana Ranch, which is located in the semi-arid zone of the Coastal Province in Kenya. Besides the domesticated wild herbivores, cattle (*Bos indicus*), camels (*Camelus dromedarius*, sheep (*Ovis aries*), goats (*Capra hircus*) and a large variety of free-ranging wild herbivores are present (King and Heath, 1975; Paling et al., 1979).

A study was conducted on the infectious diseases in domesticated wild herbivores and domestic herbivores after 7 yr of close interaction. Results of surveys for bacterial diseases are reported in this paper; those for viral diseases are reported in Paling et al. (1979).

MATERIALS AND METHODS

The study area in the Coastal Province of Kenya was described previously (Paling et al., 1979). Samples were collected from all animals, except buffalo, while physically restrained. Buffalo were immobilized by intramuscular (i.m.) injection of 0.24 mg/kg xylazine hydrochloride (Rompun-Bayer, Leverkusen, Federal Republic of Germany). Blood was collected and centrifuged in 10 ml vacuum tubes. Serum was frozen and kept at −20 C until used for testing. Vaginal and preputial secretions were collected on sterile cotton swabs and placed into 5 ml of semi-solid agar, which served as a transport medium. Nasal secretions were collected on sterile cotton swabs which were placed into 3 ml volumes of *Mycoplasma* sp. medium (MacOwan and Minette, 1976) for transport at room temperature.

Sera were tested for antibody to *Brucella* spp. by the serum (tube) agglutination test (SAT), the Rose Bengal plate test (RBPT) and the microtiter complement fixation test (CFT) as described by Philpott and Auko (1972). A titer of

TABLE 1. Results of serological tests to *Brucella* spp. in domesticated wild and domestic herbivores at Galana Ranch (1976–1977).

Species	Number reactors/ number tested	Range of titers		Number positive RBPT[c]
		SAT[a]	CFT[b]	
Buffalo	0/4	—	—	—
Eland	2/14 pos.	53	4/5	—
	3/14 susp.	50	—	—
Oryx	1/20 pos.	53	—	—
Cattle[d]	12/69 pos.	53–200	2/5–4/80	10
	7/69 susp.	50	2/2.5–1/5	—
Camel	8/102 pos.	53	3/5–3/20	2
	6/102 susp.	50	4/2.5	—
Goat	0/18	—	—	—
Sheep	0/10	—	—	—

[a] SAT, serum (tube) agglutination test.
[b] CFT, microtiter complement fixation test.
[c] RBPT, Rose Bengal plate test.
[d] Vaccinated with *B. abortus* strain 19 at 8 mo of age.

50 IU/ml in the SAT or 50% fixation at dilution 1:2.5 (2/2.5) to 25% fixation at dilution 1:5 (1/5) in the CFT was considered as suspicious. If the serum reacted positive in the RBPT or the SAT reacted at a titer of more than 50 IU, or the CFT reacted at a titer of 25% fixation at dilution 1:5 (1/5) or higher (2/5, 3/5, 4/5, 1/10, 2/10, etc.) the animal was classified as a positive reactor. Serological tests did not permit a distinction between antibody to *B. abortus* or *B. melitensis*.

Sera were tested for antibody to *Mycobacterium paratuberculosis* by a CFT modified from that described by Hole (1952). Modifications included the use of a 50% hemolytic end point while diagnostic end points were calculated by reference to a previously established minimal hemolytic dose of 50% rather than comparison with known negative and positive sera. Complete fixation with a serum dilution of 1:10 was regarded as positive.

Sera were tested by CFT for antibody to *Mycoplasma mycoides mycoides* as described for *Brucella* spp., except that the first dilution was 1:10. A reaction at a serum dilution of 1:20 or greater was accepted as evidence of exposure.

Sera were tested by CFT for antibody to *Mycoplasma* sp. (strain F38) as described by MacOwan (1976). CFT titers of 1:32 or greater were regarded as evidence of exposure.

Isolation of *Brucella* spp. was performed as described by Alton and Jones (1967). Bacteria were identified according to the methods described by Cowan (1974).

Goat digest medium (MacOwan and Minette, 1976) was initially used for the isolation of *Mycoplasma* sp. (strain F38). Thereafter, *Mycoplasma* sp. medium was prepared in the same way but from camel tissue and serum. After vigorous shaking, the transport medium was inoculated into 3 ml volumes of broth to give 10-fold dilutions from 1×10^{-1} to 1×10^{-6} and was plated on solid medium. Inoculated media were incubated at 37 C and examined for *Mycoplasma* sp. daily for 14 days. Broth cultures were examined by dark field microscopy and plates with a binocular dissection microscope at a magnification of ×30.

Tissues containing acid-fast bacteria as determined by Ziehl-Neelson staining were cultured on Lowenstein-Jensen and Dorset slopes for the isolation of *Mycobacteria* sp. (Cowan, 1974). Bovine and avian tuberculin (Central Veterinary Laboratory, Ministry of Agriculture, Weybridge, England), containing 75,000 IU/ml and 25,000 IU/ml, respectively, were intradermally injected on the lateral side of the neck, 0.1 ml at each site. The diameters of the swellings were measured using vernier calipers after 72 hr and compared with the thickness of the surrounding skin.

RESULTS AND DISCUSSION

Brucellosis

Antibodies to *Brucella* spp. were found in eland, oryx, cattle, and camels with 14, 5, 17 and 8% positive reactors, respectively (Table 1). Abortions were not reported from the five adult female eland and the calving interval averaged 1 yr. The only positive female eland was serologically negative for *Brucella* spp. on two later samplings. *Brucella* spp. were not isolated

TABLE 2. Development of antibody titers to *Brucella* spp. in camels.

Camel number	Sex	August 1976			January 1977			March 1977			July 1977			September 1977			November 1977		
		SAT[a]	CFT[b]	RBPT[c]	SAT	CFT	RBPT	SAT	CFT	RBPT	SAT	CFT	RBPT	SAT	CFT	RBPT	SAT	CFT	RBPT
7	M	–	–	–	–	–	–	ND[d]	ND	ND	ND	ND	ND	ND	ND	ND	–	4/2.5	–
12	F	50	3/20	+	25	4/10	–	40	2/10	+	25	4/5	+	ND	ND	ND	ND	ND	ND
13	M	50	–	–	–	–	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	–	–
15	F	50	–	–	25	4/10	+	ND	ND	ND	–	–	–	ND	ND	ND	–	–	–
17	F	–	–	–	50	AC[e]	–	–	–	–	–	–	–	ND	ND	ND	–	–	–
19	F	–	–	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	4/5	–
23	F	–	–	–	–	–	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	50	3/10	–
36	F	ND	ND	ND	25	2/10	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	–	–
42	F	–	–	–	50	–	–	ND	ND	ND	–	–	–	ND	ND	ND	–	–	–
45	F	–	–	–	–	2/10	–	–	–	–	–	–	–	ND	ND	ND	–	–	–
66	F	–	–	–	–	1/10	–	17	3/5	–	–	–	–	25	4/2.5	–	25	AC	+
67	F	–	–	–	–	–	–	53	4/5	–	–	–	–	25	AC	–	–	–	–
74	F	–	–	–	50	–	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	–	–

[a] SAT, serum (tube) agglutination test.
[b] CFT, microtiter complement fixation test.
[c] RBPT, Rose Bengal plate test.
[d] ND, not performed.
[e] AC, anticomplementary.

from vaginal swabs from the positive female eland, the two female eland with suspicious serological reactions and from preputial swabs from the serologically positive male eland. Abortions were not reported from the herd of about 75 adult female oryx and the average calving interval was <1 yr. A male oryx had a positive SAT response on one sampling; 3 mo later this animal was negative with all tests for brucellosis.

The camel herd contained about 70 adult females. Reproductive rates were poor. During 12 mo, from August 1976 to July 1977, almost one-half of the females became pregnant but three abortions were reported. Only seven calves were born, of which three died during the year. During a period of 15 mo a total of 300 sera was collected from 102 camels on two to six occasions, at intervals varying from 2 to 6 mo. Eight camels (8%) reacted positively with one or more tests on ≥one occasion. Six camels (6%) had suspicious reactions on an individual test (Tables 1, 2). Percentages of reactors when all animals in the herd were tested, were 4, 9 and 5%. *Brucella* sp. has not been isolated from camels in Africa, but Gatt Rutter and Mack (1963) indicated that *B. melitensis* may infect camels in Africa. Serum antibodies against *Brucella* spp. in camels have been reported in most African countries with a substantial camel population (Burgemeister et al., 1975; Waghela et al., 1978). Percentages of reactors in these studies varied from 3 to 20%. In Kenya (Waghela et al., 1978) the percentage was 14% of 172 sera tested by the same tests as used in our study. The samples originated from northern Kenya where the camels for this study were bought. Twenty-one vaginal swabs were cultured from 10 serologically suspicious or positive camels and three camels which had aborted. Also examined were preputial swabs of one serologically suspicious male camel. *Brucella* spp. were not isolated. A camel which reacted serologically positive on three consecutive occasions was killed. Gross lesions were not observed except that most of the lymph nodes appeared to be edematous and enlarged. The reproductive tract with a 3-mo-old fetus, mammary glands with lymph nodes, liver, spleen, lung and retropharyngeal, submandibular, hepatic, mesenteric and iliac lymph nodes were cultured. *Brucella* spp. were not isolated either by culture or guinea pig inoculation.

Persistence of positive reactors in camels during 16 mo strongly suggested the presence of *Brucella* sp. in the herd on Galana Ranch. It should be noted that trypanosomiasis was common in these animals and may have been partially responsible for the poor breeding results. Camels might serve as a source of *Brucella* sp. infection for other species of ruminants and control of brucellosis in camels, by elimination of positive reactors and vaccination of young animals, may be important.

Interpretation of serological reactions in many wild animals and camels is difficult, since experimental data for the standardization of the serological tests are lacking. Among the species we studied, isolation of *Brucella* sp. from individuals with antibody titers is reported only for buffalo (Mohan and Gotts, 1970; Kaliner and Staak, 1973). Gat Rutter and Mack (1963), quoting Russian authors, summarized the usefulness of the SAT and CFT for the two-humped camel (*Camelus bactrianus*). The CFT was considered to be most reliable; the SAT should be done repeatedly to be of value.

Bovine brucellosis occurred in 1960–1970 during the start of the Galana project (King and Heath, 1975), and since then all stock have been vaccinated with the Strain 19 vaccine at 8 mo of age. Antibody titers were found in 19 of 69 (28%) cattle aged 2–5 yr. Although antibody titers in cattle were in some cases (18%) higher than generally could be expected several years after vaccination (Stableforth, 1959), there was no indication for the presence of brucellosis in the cattle, sheep and goats. Brucellosis is very common in cattle in most parts of Kenya. The latest survey (1973–

1975) indicated 5% positive reactors for Kenya, 9% for the Coastal Province and 10% for Galana Ranch (Waghela, 1977). Antibodies to *B. abortus* have been recorded at low prevalence in buffalo (Sachs et al., 1968; Mohan and Gotts, 1970; Condy and Vickers, 1972, 1976; Kaliner and Staak, 1973) and eland (Sachs et al., 1968; Condy and Vickers, 1972) in eastern and southern African countries. *Brucella abortus* has been isolated on a few occasions from buffalo (Mohan and Gotts, 1970; Kaliner and Staak, 1973; Grandwell et al., 1977) and eland (Condy and Vickers, 1972). It was suggested that these infections originated from cattle. In previous studies in Kenya, we have found antibodies in three eland (7%), nine buffalo (13%) and one oryx (5%) but *Brucella* sp. was not isolated (Food and Agriculture Organization, 1978).

At Galana Ranch, abortions in the domesticated wild herbivores were rare and the reproduction rates were high. This, combined with the absence of antibody in buffalo, the presence of only low, non-persisting titers in eland and the low prevalence of antibodies in oryx, strongly indicated that brucellosis was not a problem in these domesticated wild herbivores following control of the disease in cattle.

Paratuberculosis

Complement fixing antibody at a serum dilution of $\geq$1:10 to *M. paratuberculosis* was found in eight of 102 camels, but not in wild herbivores, cattle or sheep (Table 3). Anti-complementary reactions were found in one of 69 (1%) cattle and three of 102 (3% camels). Acid-fast bacteria were not detected in feces of two serologically positive camels. Antibody to *M. paratuberculosis* has not been found in free-ranging wild herbivores in Africa (Rankin and McDiarmid, 1969). Paratuberculosis has not been recorded in camels outside Russia (Gatt Rutter and Mack, 1963), but antibody was found in 21% of camels tested in Tunisia (Burgemeister et al., 1975). We consider that the serological reaction in the camels may be non-specific (Gilmour, 1976) but further investigation is required.

Contagious bovine pleuropneumonia

Complement fixing antibody to *M. mycoides mycoides*, the agent responsible for contagious bovine pleuropneumonia (CBPP), was found in two of 69 (3%) cattle and in seven of 102 (7%) camels (Table 4). Antibody titers ranged from 1:10 in cattle to 1:160 in one camel. Antibodies were not detected in wild herbivores, sheep and goats. The cattle were vaccinated annually with a broth attenuated vaccine (TI, EAVRO, Muguga, Kenya). Camels were sampled on various occasions. On the first test five animals had positive titers, which were not present 5 mo later. Two other animals had titers on a later test, and one camel still had the same titer (1:40) 2 mo later. Anticomplementary reactions were recorded in one of 14 (7%) eland, 14 of 102 (14%) camels, six of 10 (60%) sheep and in seven of 12 (58%) goats. Culture of nasal secretions from 33 camels including all seven serologically positive camels were negative for *M. mycoides mycoides*.

Contagious bovine pleuropneumonia was enzootic in this area in the late 1960's (King and Heath, 1975), but yearly vaccination has resulted in disappearance of the clinical disease. Absence of antibody in buffalo, eland and oryx suggests that CBPP had either not been maintained within the wild animal populations or has never been present. This confirms the findings of Shifrine and Domermuth (1967) and Shifrine et al. (1970) who recorded the absence of complement fixation antibody in 102 buffalo and 81 eland in east Africa.

Bares (1968) reported 9% low positive titers against *M. mycoides mycoides* among 236 camels from enzootic areas in Chad, but considered them to be non-specific and he regarded camels as not susceptible to CBPP. Since respiratory problems were not reported from the camels on this ranch, it is possible that the antibodies found are a result of cross-reaction with another or-

TABLE 3. Results of the complement fixation test to *Mycobacterium paratuberculosis* in domesticated wild animals and domestic animals at Galana Ranch (1976–1977).

Species	Number positive[a]/ number tested
Buffalo	0/4
Eland	0/14
Oryx	0/20
Cattle	0/69
Camel	8/102
Sheep	0/10
Goat	1/12

[a] Complement fixation at ≥1:10 was considered positive.

TABLE 4. Results of complement fixation tests to *Mycoplasma mycoides mycoides* and *Mycoplasma* sp. (strain F38) in domesticated wild animals and domestic animals at Galana Ranch (1976–1977).

Species	*M. m.* subsp. *mycoides*[a] (number positive/ number tested)	*Mycoplasma* sp. (strain F 38)[b] (number positive/ number tested)
Buffalo	0/4	3/4
Eland	0/14	0/10
Oryx	0/20	0/10
Cattle[c]	2/69	2/18
Camel	7/102	64/102
Sheep	0/10	0/10
Goat	0/12	0/16

[a] Fixation at ≥1:20 was considered positive.
[b] Fixation at ≥1:32 was considered positive.
[c] The cattle are vaccinated annually against CBPP.

ganism. Based on available results (Gatt Rutter and Mack, 1963; Bares, 1968), one should not regard camels as a potential source of *M. mycoides mycoides* for cattle until this organism has actually been isolated from a camel. There seems to be no risk of CBPP on a mixed farm as long as the disease is controlled in cattle.

Contagious caprine pleuropneumonia

Contagious caprine pleuropneumonia (CCPP) caused by *Mycoplasma* sp. (strain F38) is endemic in regions of Kenya (MacOwen and Minnette, 1976; MacOwen et al., 1977). The presence of antibody to *Mycoplasma* sp. (strain F38) in four of four buffalo and in 64 of 102 (63%) camels on this ranch has been reported (Paling et al., 1978). Antibody was found also in two of 18 (11%) cattle but not in eland, oryx, sheep and goats (Table 4). Contagious caprine pleuropneumonia has not been diagnosed in sheep and goats on this ranch. The highest titer was recorded in buffalo (1:2,048). A portion of the camel herd was tested on four occasions (Table 5). The highest percentage of positive sera as well as the highest individual titers were recorded in July, 1977. The longest period antibody was present in an individual was 11 mo. Antibodies were found in camels of all ages.

Attempts were made to isolate *Mycoplasma* spp. from nasal secretions. On four occasions a total of 47 nasal swabs was collected from 33 camels, of which 30 were or had been serologically positive. Nasal swabs were taken also from four buffalo, of which three were serologically positive. Structures resembling *Mycoplasma* sp. colonies were observed on three occasions on primary culture of camel samples using goat digest medium, but subcultures remained sterile. Similar results were obtained using *Mycoplasma* sp. medium prepared from camel tissue and serum.

A high proportion of buffalo and camels studied had complement fixing antibodies to *Mycoplasma* sp. (strain F38). However, failure to grow *Mycoplasma* spp. from nasal secretion indicated a need for isolation techniques suitable for this organism in these hosts. Although camel *Mycoplasma* sp. medium was prepared from camel serum free of complement fixing antibody, possibly the techniques were not sufficiently sensitive to detect very small numbers of the agent. Alternatively, *Mycoplasma* spp. may have been present at sites other than nasal mucosa in the camels. It is possible also that the CFT indicated previous infection with a related *Mycoplasma* sp., or another organism with antigens in common with *Mycoplasma* sp. (strain F38). The organism has been present in the camel herd for at least 1 yr, since 4-fold differences in individual titers were present

TABLE 5. Incidence of complement fixing antibody titers to *Mycoplasma* sp. (strain F38) in camels on Galana Ranch (1976–1977).

Titers[a]	August 1976		January 1977		March 1977		July 1977	
	n	%	*n*	%	*n*	%	*n*	%
Below 32	20	33	50	59	18	69	0	—
32 and 32–64	16	27	19	22	3	11	0	—
64 and 64–128	18	30	13	15	3	11	0	—
128 and 128–256	2	3	3	4	2	8	3	25
256 and 256–512	2	3	—	—	—	—	4	33
512 and 512–1,024	2	3	—	—	—	—	1	8
Over 1,024	—	—	—	—	—	—	4	33
Total number tested	60		85		26		12	

[a] Complement fixation $<1{:}32$ was considered negative; $\geq 1{:}32$ was considered positive.

on each sampling occasion. There was no clinical disease in buffalo and camels at the time of sampling or in goats which were present in the same area as the buffalo. There was no direct contact between the buffalo and the camels. Other isolation methods should be developed for this organism and transmission experiments should be undertaken to establish if buffalo and camels are susceptible to infection with *Mycoplasma* sp. (strain F38). Buffalo and camels should be considered as possibly susceptible to *Mycoplasma* sp. (strain F38), but their role in the epizootiology of CCPP is not clear.

Tuberculosis in camels

Eighteen male and 23 female camels, were intradermally injected at separate sites with 0.1 ml doses of bovine and avian tuberculin. Thirty-three percent of the males and 13% of the females reacted to bovine tuberculin and 61% of the males and 13% of the females reacted to avian tuberculin.

Eight of the nine positive reactors to bovine tuberculin also reacted to avian tuberculin with seven having a more severe reaction. Skin thickness increase caused by bovine tuberculin ranged from 0.5 to 3.1 cm with only one animal having an increase of more than 2.5 cm. Skin thickness increase caused by avian tuberculin ranged from 2.1 to 4.9 cm including 10 animals with swellings larger than 2.5 cm.

One camel killed because it was a brucellosis reactor had small areas of fibrosis and mineralization in the lung. Impression smears of these tissues, stained by the Ziehl-Neelson method, contained acid-fast bacteria. However, attempts to culture *Mycobacteria* spp. were unsuccessful. Histopathologic examination of the lesions revealed partly mineralized granulomas containing acid-fast bacilli. The liver contained lymphoid nodules and areas of necrosis surrounded by epithelioid and giant cells.

References to intradermal testing of camels with tuberculin could not be found, but finding 15 of 41 (37%) reactors and acid-fast bacteria causing granulomas in one animal indicated the presence of a mycobacterial disease. Only the bovine tubercle bacillus (*Mycobacteria bovis*) has been isolated from camels (Mohan and Gotts, 1970). Because bovine tuberculosis is uncommon in cattle in Kenya, the possibility of human tuberculosis which is present among the nomadic tribes of northern Kenya or avian tuberculosis, so far identified only among wild birds (Cooper et al., 1975), could not be excluded. Precautions should be taken to prevent transmission to humans and susceptible herbivores like buffalo (Rankin and McDiarmid, 1968; Mohan and Gotts, 1970) and oryx (Lomme et al., 1976).

Skin necrosis in camels

A lesion described as contagious skin necrosis (Gatt Rutter and Mack, 1963; Richard, 1975) was observed in 10 of 102 (10%) camels. Lesions, in the form of swellings occurred subcutaneously on the neck, thorax or abdomen and developed into abcesses which later ulcerated. Microfilaria were not detected in the blood of these animals and microscopic examination of affected tissues did not indicate the presence of filaria. On three occasions exudate was cultured and *Escherichia coli, Streptococcus bovis, Staphylococcus epidermis* and *S. aureus* were isolated. *Streptococcus* spp. were isolated also by Richard (1975) but the ethiology remains obscure. Similar lesions were not recorded in any other species which were in contact with the camels.

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