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SURVIVAL OF HAEMOPHILUS SOMNUS IN BIOLOGICAL MILIEUS

K. Serdar DİKER (*) Hakan YARDIMCI (*) Kazunori HASHIMOTO (**)

INTRODUCTION

Haemophilus somnus is the aetiological agent of several diseases of cattle. This small, gram-negative plemorphic cocobacillus is involved in a variety of syndromes including thromboembolic meningoencephalitis (TEME) (20), acute respiratory disease (4, 8), reproductive disease (7, 17), mastitis (2) and abortion (13). Not only is this organism an important pathogen but, also many apparently healthy cattle carry it on their nasal, preputial and vaginal mucosa (5, 12, 16). High agglutination titers against H.somnus have also been reported in normal cattle (1).

Although H.somnus has been shown experimentally to reproduce clinical syndromes in cattle when given intravenously, intratracheally or intra uterine (9, 18, 21), the source of organism and the route of transmission of the infection between hosts in natural environment are poorly understood. Bovine secretions and excretions contaminated with H.somnus are most likely important sources in transmission of infection caused by this organism. We therefore performed in vitro experiments to study the behavior of H.somnus in several environments and physiological milieus.

MATERIALS AND METHODS

Bacterial strains and media: In this study we used two strains of H.somnus. 1) H.somnus strain 43826, isolated from the brain of a steer with naturally occuring TEME was supplied from S.C.Groom, University of Guelph, Ontario, Canada. 2) H.somnus strain BV14

(*) Department of Microbiology, Veterinary Faculty, Ankara University, Ankara 06110, TURKEY
(**) Nippon Zenyaku Kogyo Ltd, JAPAN

was originally isolated from the vagina of an apparently healthy cattle. Bacteria were grown on brain-heart infusion agar containing 7% sheep blood, 0.5% yeast extract and 0.1% thiamine monophosphate (BHIBYT agar) in all experiments. For preservation, H. somnus strains were inoculated into the yolk sacs of 6 days-old embryonating eggs. After 24 h incubation, the yolk sacs were harvested and stored at —70°C.

Milieus tested: Defibrinated blood, serum, bile, milk, urine and vaginal mucus samples were collected as aseptically as possible from clinically H.somnus free cattle which had not received antimicrobial agents. Blood and bile samples were tested for sterility by culturing, serum and urine samples passed through a 0.2 micrometer membrane filter and milk and vaginal mucus were autoclaved for 15 min for sterilizing prior to use in the experiments.

Experiments of survival: Before each experiment, bacteria were obtained from frozen storage and plated onto BHIBYT agar. After 24 h incubation at 37°C in 10% CO_2 and 90% air, the growth was suspended in sterile phosphate buffered saline (pH. 7,3). Multiple sterile aliquots of excretions and secretions (9 ml each) were inoculated with bacterial suspension (1 ml each) at time zero and kept at 4°C, 25°C and 37°C. Initial bacterial counts were made and repeated daily for the ten days, and weekly for the rest up to 59 days. This method for determining sampling intervals was used for all milieus tested. If contamination of sample was observed, we then continued our counts with an unopened sample which had been also inoculated at time zero and kept at the same temperature.

Bacterial counts: For determining initial and subsequent counts (CFU/ml), samples were withdrawn from the test milieus. For each test milieus for each time, 0.1 ml was withdrawn and serial 10-fold dilutions were inoculated on duplicate BHIBYT agar plates according to the Miles-Misra method (15), and incubated in a carboxyphilic atmosphere at 37°C for 48 h. Following examination, the resulting colonies were determined as H.somnus if they satisfied the criteria previously outlined (11).

RESULTS

The viability of two H.somnus strains in biological milieus at different temperatures is depicited in Table 1. The survival of H. somnus was strain, temperature or time dependent. Log_{10} numbers of microorganisms mixed with blood, serum, milk, vaginal mucus,

bile and urine are shown in Figures from 1 to 6, respectively. H. somnus strain 43826 survived for more than 59 days in blood and serum at 37°C. Strain BV14 survived for 3-6 days in the same condition. This strain survived longest (24 days) in vaginal mucus at 37°C. Survival was four days or less in all cases when the organisms were mixed with bile or urine.

TABLE 1. The influence of biological milieus on H.somnus viability at different temperatures.

	Survival of H.somnus (days)					
	Strain 43826			Strain BV14		
Milieus tested	4°C	25°C	37°C	4°C	25°C	37°C
Blood	9	52	>59	6	3	6
Serum	10	17	>59	6	3	3
Milk	5	3	5	17	9	17
Vaginal mucus	4	2	2	9	6	24
Bile	2	3	<1	3	2	<1
Urine	2	2	<1	4	3	3

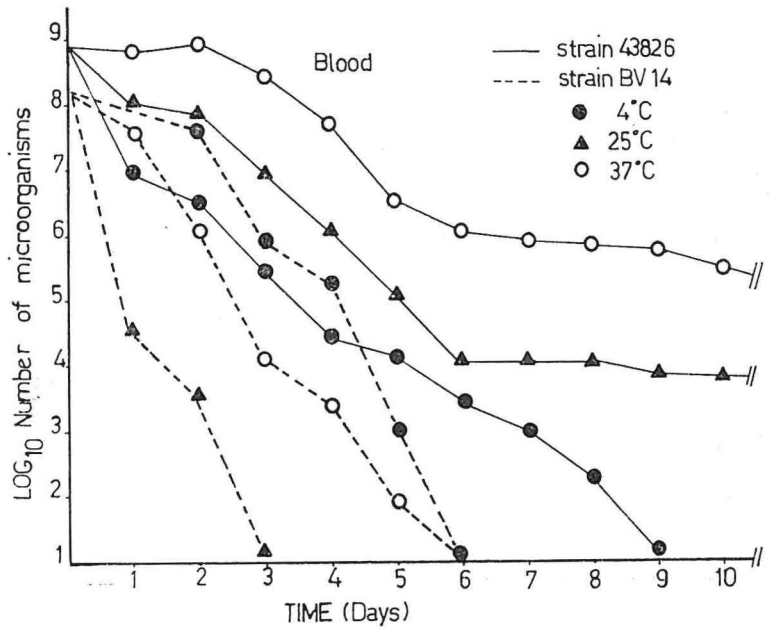

FIGURE 1. Survival of two strains of H.somnus in blood kept at 4°C, 25°C, 37°C

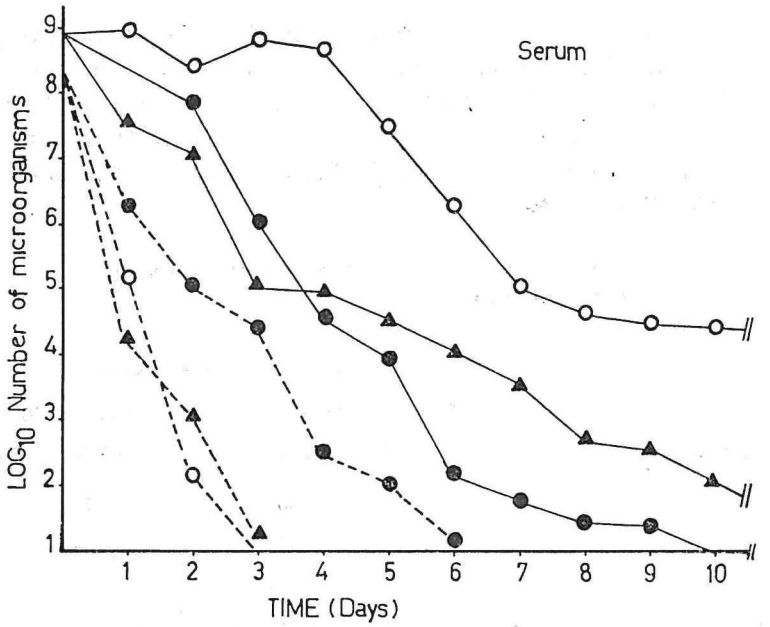

FIGURE 2. Survival of two strains of H.somnus in serum kept at 4°C, 25°C, 37°C

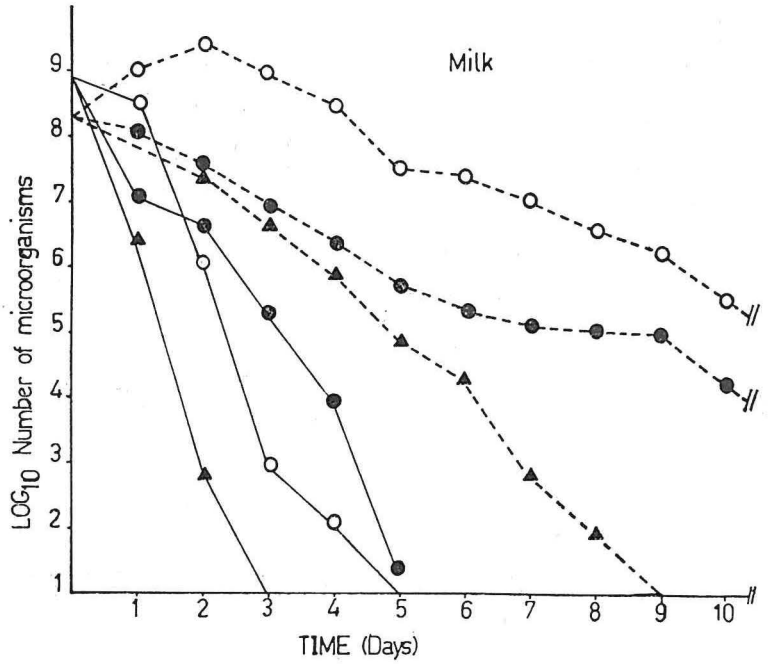

FIGURE 3. Survival of two strains of H.somnus in milk kept at 4°C, 25°, 37°C

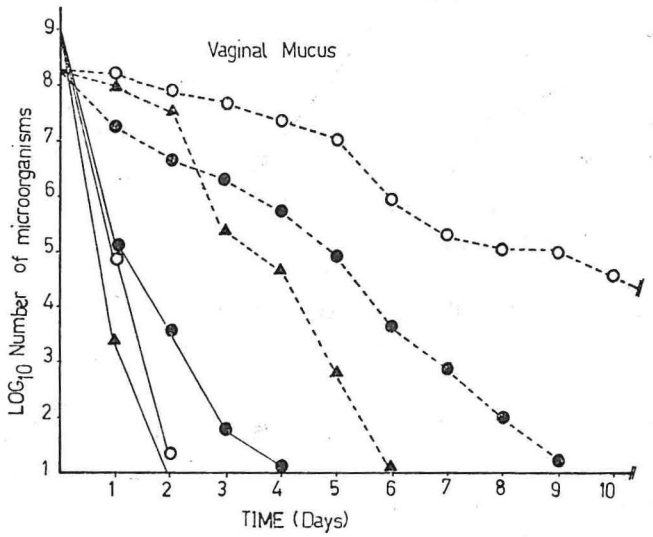

FIGURE 4. Survival of two strains of H.somnus in vaginal mucus kept at 4°C, 25°C, 37°C

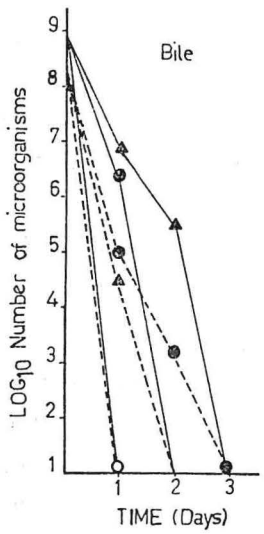

FIGURE 5. Survival of two strains of H. somnus in bile kept at 4°C, 25°C, 37°C

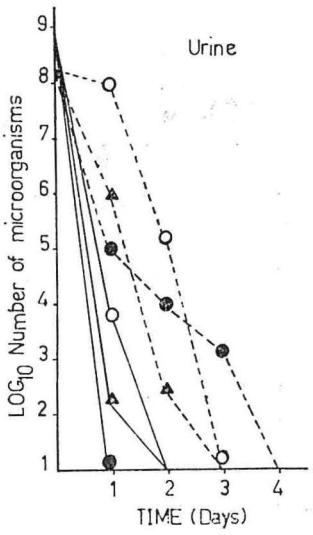

FIGURE 6. Survival of two strains of H. somnus in urine kept at 4°C, 25°C, 37°C

DISCUSSION

H. somnus is known to be a significant pathogen for cattle. Despite recent advances, the epidemiology and pathophysiology of H. somnus infection are not fully defined.

In these experiments we used laboratory strains of H.somnus to evaluate survival of this organism in several physiological milieus. Although these strains were minimally passaged, adaptation to artificial media may have affected the results we obtained, and these results may not be identical to those seen in nature with wild type strains.

H.somnus strain 43826, a clinical isolate from TEME, survived well in blood and serum at 25°C and 37°C. Whole blood mixed with this strain contained 10^4—10^6 organisms per ml after ten days. Dewey and Little (6) have obtained very similar results with the same strain. The survival of vaginal strain was very short when comparing with the clinical isolate. Several investigators have showed that all isolates of H.somnus from clinical cases were serum resistant (3, 19). They have also suggested that there is a strong correlation between virulence and serum resistance. This phenomenon may explain the difference between the behavior of our two strains.

H.somnus has been isolated from genital tract of normal cattle (12, 16) and several reports described the genital tract infection in cattle caused by this organism (7, 17). In our study, survival of vaginal strain in vaginal mucus was well at 37°C. Survival of vaginal strain in vaginal mucus for a long time suggests that infected vaginal mucus from animals might be a potential source of environmental contamination. Little (14) has also pointed out the importance of the genital tract as the main ecological niche of this organism.

H.somnus has been isolated from bovine mastitis (2) and mastitis has been experimentally induced in cows (10). In this study, milk mixed with vaginal H.somnus strain contained 10^4—10^5 organisms per ml after ten days at 25°C and 37°C. Our data showed that H.somnus can survive in milk in environment to contaminate another animal.

H.somnus organisms have been isolated from urine of cattle in natural and experimental cases of TEME (20). It has also been suggested that this source may represent a significant factor in the transmission of H.somnus associated disease (11). In our study, however, survival of both strains in urine was short for all temperatures. Dewey and Little have also found that H.somnus survived for less than two hours in urine (6). These data show that urine is not a potential source of environmental contamination for long periods. On the other hand, it must be kept in mind that discharge of urine onto hard surfaces cause aerolization and this may be inhaled by cattle in close proximity.

The viability of our strains was very short in bile. Log_{10} numbers of organisms decreased rapidly in this milieus at all temperatures. Sensitivity of our strains to bile shows that bile has no role in the pathogenesis of H. somnus infection.

In general, strain 43826 survived longer than strain BV14 in blood and serum, whereas viability of BV14 in vaginal mucus and milk was longer than those of strain 43826. It is most probable that several H.somnus biotypes are present in the bovine environment and these strains have an affinity to particular sites in body.

SUMMARY

Survival of two Haemophilus strains in blood, serum, vaginal mucus, milk, urine and bile at different temperatures was investigated. H.somnus strain isolated from a clinical case survived for more than 59 days in blood and serum at 37°C. H.somnus isolated from normal vaginal flora survived longest (24 days) in vaginal mucus at 37°C. Survival was four days or less in all cases when the organisms were mixed with bile and urine.

ÖZET

HAEMOPHILUS SOMNUS'UN BİYOLOJİK MATERYALDEKİ DAYANIKLILIĞI

Sığır orijinli iki Haemophilus somnus suşunun kan, serum, vaginal mukus, süt, idrar ve safra içinde 4°C, 25°C ve 37°C'de canlı kalma süreleri incelendi. İnfeksiyon kökenli suş 37°C'de kan ve serumda 59 günden fazla canlı kaldı. Vaginal floradan izole edilen suş ise en uzun süre 37°C'deki vaginal mukusta canlılığını korudu. Her iki suş incelenen tüm ısılarda, safra ve idrarda kısa sürede öldü.

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