

*During a survey to determine bacteriophage types of staphylococci in the milk of dairy cattle on Oahu, antibiotic resistant S. aureus Type 80/81 was found. The same type was found in several human beings associated with the dairy. Theories as to the origin of the infection are presented, and the public health significance of the findings is discussed.*

## **BACTERIOPHAGE TYPE 80/81 STAPHYLOCOCCAL INFECTION IN HUMAN BEINGS ASSOCIATED WITH MASTITIS IN DAIRY CATTLE**



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A SURVEY was conducted to determine the bacteriophage types of staphylococci present in the milk of dairy cattle on the island of Oahu, Hawaii, and whether these types are common to those found in staphylococcal infections of human beings. During the survey antibiotic-resistant strains of *Staphylococcus aureus*, phage Type 80/81, were found in the milk of four cows with mastitis, three in one dairy herd. Further investigations revealed that five more cows and one of 15 employees at this dairy, as well as two members of his family, were infected with Type 80/81 staphylococcus.

A report of preliminary findings has already been published.<sup>1</sup> The following report gives further information and data obtained from the completed investigation at the dairy.

### **Materials and Methods**

#### **Selection and Collection of Specimens**

The dairy had approximately 1,000 head of cattle and was milking approximately 800 of these. It was obvious that milk from all cows could not be

cultured so milk samples from 108 cows showing evidence of mastitis either grossly or by the California Mastitis Test<sup>2</sup> were selected for the initial studies.

There were two large vats at the dairy where milk was stored after cooling prior to transportation by tank trucks to a processing plant. One tank contained the milk obtained from approximately 125 Guernsey cows at one milking; the other, the milk of approximately 650 Holstein cows. Twenty-seven 20-ml samples of bulk milk, both agitated and unagitated, were collected at separate times from these vats.

Milk cultured during the investigation of the herd was obtained by a veterinarian, a dairy inspector, or the dairy herdsman. The following techniques were used to collect milk from individual cows: Udders and teats were cleansed with a 1:1,000 solution of chlorohexidine diacetate (Winthrop), dried with individual paper towels, and approximately 10 ml of fore milk were withdrawn from each quarter into a sterile bottle. In some instances, individual quarter samples were obtained. In most instances milk samples were

not refrigerated because they were transported to the laboratory shortly after collection.

Human specimens were obtained by one of the authors or a hospital laboratory technician. Nasal and throat cultures were taken with dry sterile swabs and furuncle exudate was taken by aspiration with a sterile syringe and needle.

At the outset of the investigation there were 11 men employed by the dairy who had direct contact with the cows. Swab samples were obtained from the anterior nares of these men and they were questioned about current or past infections indicative of staphylococcal origin. Six of the men were found to have paronychia on one or more fingers, which was attributed by the dairy manager to the frequent contact with disinfectant solution (chlorohexidine diacetate) during the milking operation. Specimens from finger lesions were collected on sterile cotton swabs soaked in physiological saline solution.

Only one employee gave a history of a frequent occurrence of furuncles or other disease that could be attributable to staphylococcal infection. This man had a large draining furuncle on his back at the waistline, approximately three inches lateral to the spinal column. Exudate was collected from this lesion on the same day the nasal swabs were taken. In addition, samples of disinfectant solutions used in the milking parlor and swabs from hose nozzles frequently handled by the employees were taken for bacteriological examination. Subsequently, additional specimens from the original dairy employees, new employees, and the family of an employee previously found to be infected with *Staphylococcus aureus*, phage Type 80/81, were cultured.

#### Laboratory Methods

With a few exceptions, milk collected during the survey and during the in-

vestigation at the one dairy was sent to the Hawaii State Veterinary Laboratory, incubated in 37° C for 12 hours, and then streaked onto blood agar plates. Blood agar plates were used to permit isolation and studies of organisms other than staphylococci, particularly streptococci and actinomyces. After 12 to 24 hours, colonies resembling staphylococci were isolated and coagulase activity was tested by the tube method using human plasma. Coagulase-positive cultures were then sent to the central laboratory of the Hawaii State Health Department for confirmation, bacteriophage typing, and antibiotic sensitivity tests.

Milk samples which were collected from bulk storage tanks and also from a few cows were submitted to the State Health Department Laboratory where media selective for staphylococci were employed for isolation. In these instances 5 ml of well mixed milk were added to 5 ml of double-strength trypticase soy broth containing 13 per cent sodium chloride to provide a final concentration of approximately 6½ per cent sodium chloride. After incubation overnight at 37° C, a loopful was streaked onto mannitol salt agar (Difco) plate which was then incubated at 37° C overnight. Staphylococcal colonies surrounded by yellow zones were fished onto trypticase soy agar (BBL) slants. Swabs from nose, throat, lesion, and so on, were streaked onto mannitol salt agar for the isolation of staphylococci.

Coagulase-positive, mannitol-fermenting staphylococci were bacteriophage typed by the method described by Blair and Carr<sup>3</sup> using the following phages: 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 73, 75, 77, 83, 42D, 44A, 81, and 187.

Antibiotic sensitivity of staphylococcus strains was determined by using Difco unidiscs and BBL sensi-discs of low concentrations only.

## Results

Table 1 shows the source, distribution, and predominate phage types of *S. aureus* found at the dairy and the frequency of isolation. It is noteworthy that *S. aureus* was found in the milk of 50 per cent of the cows tested, in 100 per cent of the bulk milk samples, and in 47 per cent of the human beings tested. Undoubtedly, the percentage of cows with staphylococci in milk would have been much higher if media selective for the isolation of staphylococci were used throughout the study. Quite frequently, growth of staphylococci on blood agar plates was obscured by overgrowth of other organisms.

As shown in Table 2, Type 80/81 *S. aureus* was isolated in May from the employee's anterior nares and also from a furuncle on his back. Although not shown in Table 2, Type 80/81 was isolated from nares of both members

of his family in June. The isolation made in August was from another furuncle on the same employee. He had previously received two intensive courses of therapy with antibiotics, to which the 80/81 strains were found sensitive, and had two negative nose and throat cultures and a complete clearing of the lesion before he was allowed to return to work with the cattle in July.

The three cows found shedding staphylococcus phage Type 80/81 in March were discovered during the general survey of dairies. These cows were removed from the herd. Another sampling of milk from 108 cows in the same herd in May revealed Type 80/81 in milk of three additional cows. These cows were also segregated from the rest of the herd. During June, July, and August laboratory services were limited to examinations of bulk milk samples and specimens from employees showing evidence of staphylococcal infections.

Table 1—Source and Frequency, Including Predominate Phage Types, of *Staphylococcus aureus* Isolated at the Dairy

	Human		Cows		Bulk Milk Tanks		Fomites
	No.	Per cent	No.	Per cent	No.	Per cent	
Individuals sampled	17		306		2		10
Specimens collected	54		795		27		10
Positive specimens	19	35	168	21	27	100	0
Individuals with <i>Staphylococcus aureus</i>	8	47	154	50	2		
Isolates phage typable	14	74	94	56	16	47	
Predominant phage Types:							
80/81	5		11		2		
3A	5						
52A/79	2						
42D			21		6		
29/52/52A/79/80/3A/3B/3C/ 55/71/6/7/42E/47/53/54/73/ 75/77/83/42D/44A/187/81			14				
47/42E/47/42B/42D/44A					5		

**Table 2—Source and Frequency of Staphylococcus Phage Type 80/81 Isolated at the Dairy**

	Cows	Human	Bulk Milk Tanks	
			Guernsey Milk	Holstein Milk
March	3/18*			
April				
May	3/108	1/11*		
June	0/7	0/4		
July	0/24	0/2	2/5†	0/6
August		1/1		
September	2/161	0/14		
October	0/56		0/2	0/2
November	0/60		0/3	0/3
December			0/3	

\* Denominator—No. of individuals sampled; numerator—No. of individuals infected with Type 80/81.

† Denominator—No. of samples collected; numerator—No. of samples containing Type 80/81.

Two out of five bulk milk samples collected at different times in July from the Guernsey milk storage tank contained staphylococcus Type 80/81. Since five of the six cows infected with Type 80/81 were Guernseys and the Guernsey bulk milk contained Type 80/81, it was considered important to culture milk samples from each cow in the Guernsey herd. This was done in September. Some of the Guernsey cows showing gross evidence of mastitis were sampled two or three times and some Holstein cows with acute mastitis were also sampled. Two more Guernseys were found to be shedding staphylococcus Type 80/81 in their milk, bringing the total infected with this type to eight, one Holstein and seven Guernseys.

Milk samples from all Guernsey cows that had been sampled only once previously were collected and cultured in October and November with negative results. Staphylococcus Type 80/81 was not detected in bulk milk samples collected through December. Since then there has been no additional evidence of staphylococcus 80/81 infections

occurring at the dairy. Although routine sampling has not been performed, milk samples from acute cases of mastitis at the dairy are still submitted to the Veterinary Diagnostic Laboratory and staphylococcus cultures are forwarded to the Health Department for phage typing. The dairy management agreed to send any employee with evidence of staphylococcal disease to a physician for diagnosis and treatment.

#### Comparison of Bacteriophage Types

The predominant bacteriophage types of staphylococcus isolated from all sources are listed in Table 1. The percentage of strains typable was considerably higher from human sources than from animals. Type 80/81 was the only type found common to both animals and man at the dairy. Type 52/52A/80 was isolated from six cows with mastitis, and, though not found in the dairy employees, unpublished data at the State Health Department indicate this type is a common cause of human staphylococcal infection in Hawaii.

**Resistance to Antibiotics**

Phage Type 80/81 was the only type consistently resistant to several antibiotics when tested *in vitro*. A large number of nontypable strains of coagulase-positive staphylococcus isolated from cows were found to be resistant to streptomycin and have some resistance to penicillin.

The antibiograms of most of the human and cattle 80/81 strains at the dairy had similar patterns—resistance to chlortetracycline, oxytetracycline, tetracycline, and dihydrostreptomycin; variable resistance to penicillin; and susceptible to chloramphenicol, oleandomycin, bacitracin, neomycin, carbomycin, and novobiocin.

**Persistence of Type 80/81 in Cows**

Before additional studies could be made, the first two of the eight cows shedding phage Type 80/81 were slaughtered because of poor milk production. The other six were observed

for periods from four to 12 weeks following the first isolation of Type 80/81 (Table 3). Type 80/81 was isolated from the milk of Cow A on the sixth week after the first isolation and on the fifth week in two others, C and E. Cow C had received two courses of neomycin by intramammary infusion one and two weeks after the initial isolation. Milk samples from Cows E and F were collected and cultured periodically for 12 weeks after the initial isolation and only one additional recovery of Type 80/81 was made.

Calves were allowed to nurse Cows E and F during the 12-week observation period. The calves remained well, and the cows were returned to the dairy since they had remained negative on culture and the mastitis had subsided.

**Pathology in Cattle**

All of the cows shedding staphylococcus Type 80/81 had some degree of udder abnormality, varying from slight

**Table 3—Persistence of Staphylococcus Phage Type 80/81 in the Milk of Dairy Cows**

No. of Weeks Since First Isolation of 80/81	Cows Found Shedding Type 80/81					
	A	B	C	D	E	F
1	Neg.		+(T)‡	Neg. (S)	Neg.	Neg.
2	Neg.		(T)			
3		Neg.			Neg.	Neg.
4		Neg. (S)†	Neg.			
5			+		+	Neg.
6	+					
7	(S)*				Neg.	Neg.
8						
9					Neg.	Neg.
10						
11						
12			Neg. Neg. (S)*		Neg.	Neg.

\* (S)—Slaughtered.

† Neg. (S)—Type 80/81 not isolated from mammary gland tissue collected at slaughter.

‡ (T)—Intramammary infusion of neomycin.

**Table 4—Phage Type, Source and Frequency of *Staphylococcus aureus* Isolated from a Family Associated with the Dairy**

Date	Mr. K.			Mrs. K.			H.K.	
	Nose	Throat	Boil	Nose	Throat	Boil	Nose	Throat
May 15, 1959	80/81		80/81 (T)					
June 4, 1959	Neg.	Neg.		Neg.	Neg.	80/81 (T)		80/81 (T)
June 23, 1959	Neg.	Neg.		3A	3A		3A	Neg.
Aug. 10, 1959			80/81 (T)					
Sept. 9, 1959	Neg.			3A			3A	3A
April 5, 1960	Neg.	Neg.		3A	Neg.		Neg.	3A

(T)—Treatment with selected antibiotics.

induration to severe, acute, and chronic inflammation in all quarters with extensive destruction of glandular tissue. The microscopic examination of tissue sections revealed in two cases a purulent mastitis characterized by foci of neutrophils, active and necrotic, a ballooning degeneration of the lactiferous epithelium, and increased fibroblastic activity of the septal tissue. In general, the pathology was typical of mastitis associated with other types of *S. aureus* and there was insufficient clinical or pathological evidence to suggest a more serious infection than those associated with other types.

#### Findings in Employee and Family

Table 4 outlines the bacteriological findings in the dairy employee, his wife, and 18-month-old son. The father had a history of frequent experiences with boils and sore throats during the nine-month period before the investigation, commencing approximately three months after his initial employment by the dairy. His primary duties had been in the hospital barn where all sick or diseased animals were treated, including those with mastitis. He assisted the herdsman with treatment of the cows. His wife was afflicted with a boil, and

his son had been experiencing a chronic sore throat for approximately three months, which apparently had been unresponsive to medical treatment.

A course of triacetyloleandomycin given in May followed in two weeks by a course of chloramphenicol was apparently effective in controlling the staphylococcal infection in the dairy employee for at least two months. He had another furuncle in August from which staphylococcus Type 80/81 was isolated. Another course of chloramphenicol was administered at that time and he has not shown further evidence of staphylococcal infection.

It is interesting to note that, after his wife and son were given chloramphenicol, staphylococcus Type 80/81 was not again detected. They persisted in carrying antibiotic-sensitive Type 3A which was initially found in the second specimens taken.

#### Discussion and Conclusion

##### Possibility of Cattle Infecting Human Beings

The available information allows only speculation as to the original source of staphylococcus Type 80/81 infection in the dairy herd studied. Coagulase-positive staphylococci were found to be

rather common in mastitic secretions of a large number of cows in the dairy herd under investigation. Although adequate records are not kept on individual animals by the dairy management, it was learned that, prior to the discovery of Type 80/81 staphylococci at the dairy, a wide variety of antibiotics had been used by intramammary infusion for the treatment of cows with mastitis. Although unlikely, it is possible that the resistant strains developed in bovine mammary glands and are epidemiologically unrelated to the strains of staphylococci isolated from the dairyman and his family. It is also possible that the human staphylococcal infection was contracted from the cows. The infected employee spent most of his working hours in the hospital barn where cows, including the ones found to be secreting Type 80/81 staphylococci, were housed for treatment of mastitis and other ailments.

Further circumstantial evidence that he may have been infected from the cows is that he or his family had shown no recurrent or obvious symptoms of staphylococcal disease prior to his employment by the dairy. After initial therapy, swabs from his nose and throat, although limited in number, were negative and he was symptom-free until he again came into contact with the dairy cattle. His family was apparently freed of staphylococcus Type 80/81 after initial therapy which, if such was the case, has eliminated the source of his reinfection. Admittedly, there was not enough culturing done to say with any degree of certainty that the dairy employee or his family were not harboring Type 80/81 in some part of their anatomy.

#### Possibility of a Human Being Infecting Cows

The third possibility is that the dairyman, or one of his family, contracted Type 80/81 infection from an outside source and then infected the cows. Ex-

cept during the birth of his child 18 months previously, none of the family had a history of recent hospitalization. The hospital where the child was born (nine months before symptoms of staphylococcal disease were evident in the father or mother) had not been known to have a staphylococcal disease problem. This, however, does not preclude the possibilities either that the child or his mother could have acquired the organism in the hospital and remained asymptomatic carriers or that the family acquired the infection in the community.

Phage Type 80/81 has been the most predominant type among the cultures submitted by physicians and hospitals to the State Health Department Laboratory for bacteriophage typing. During 1958, Type 80/81 accounted for 30 per cent of coagulase-positive strains submitted, and in 1959, 21 per cent.<sup>4</sup> The next most frequent type found was Type 7 for both years, 5.5 and 5.9 per cent, respectively.<sup>5</sup> Twenty-six per cent of Type 80/81 cultures were submitted by the various clinics and physicians and therefore were obtained presumably from nonhospitalized individuals. These findings were highly indicative of community infection, which is well documented in other areas.<sup>6</sup>

#### Communicability and Virulence of Type 80/81

Varying opinions appear in the literature concerning the communicability and virulence of staphylococcus Type 80/81 as compared with other staphylococci. Blair<sup>7</sup> states: "There appears to be little question that staphylococcus of Type 80/81 probably possess enhanced virulence and a high degree of communicability . . . and is capable of producing a variety of clinical forms of staphylococcal disease." Wentworth<sup>8</sup> and associates describe family infections persisting for as long as four years and requiring extensive medical care and hospitalization. The cost to one family

for antibiotics and medical care, exclusive of hospitalization, was estimated in excess of \$1,500. Spink<sup>9</sup> provides evidence that infections with antibiotic-resistant strains present more difficult therapeutic problems and appear to be causing a higher case fatality rate than infections with sensitive strains. Anderson<sup>10</sup> and Dowling<sup>11</sup> in separate reports recognized the seriousness of the antibiotic-resistant strains but pointed out the need for more information and better methods of determining invasiveness, pathogenicity, and virulence of individual strains.

Although the communicability and virulence for cattle of the Type 80/81 strains found during the dairy investigation appeared to be no greater than those of other types of staphylococci, this has not been true in all studies. Ditchfield, in a personal communication, reported that antibiotic-resistant strains of Type 80/81 in Canada appeared to have caused a more severe mastitis in one dairy herd observed than did other types. He stated there was evidence establishing that the 80/81 staphylococcus infection in cows was contracted from a human source. Zinn<sup>12</sup> described furunculosis caused by Type 80/81 on the mammary glands of cows in one herd. The condition was intractable to treatment, and the herd eventually had to be destroyed. Here again, the bovine infections were attributed to a human source.

#### Public Health Significance

Animals, including cows, have been reported as asymptomatic carriers of antibiotic-resistant Type 80/81 staphylococci and were believed to have transmitted this type to students at a veterinary college.<sup>13</sup> Type 80/81 was isolated from the external nares of one of 22 cows and was also isolated from the milk of a cow with mastitis. Apparent infections with Type 80/81 staphylococci as

well as asymptomatic carriers in other animals, including dogs, horses, and goats, were also reported. In another report<sup>14</sup> it was inferred that dogs may be capable of harboring human epidemic strains of staphylococci and transmitting them to human beings.

It seems obvious that the finding in animals of antibiotic-resistant staphylococcal strains commonly associated with human infections presents a problem of concern to both public health and animal health officials and warrants further attention. The occurrence of Type 42D has been reported as rather common or predominating in dairy herds and has been associated with production of enterotoxin.<sup>15-17</sup> These findings reaffirm the necessity for strict enforcement of health and sanitary regulations on production, storage, and processing of milk, particularly in reference to proper refrigeration, holding, and pasteurization.

#### Summary

During a survey conducted to determine the bacteriophage types of staphylococci present in milk of dairy cattle on the island of Oahu, the milk of three cows in one herd was found to contain antibiotic resistant *S. aureus* of phage Type 80/81. One of 15 employees at this dairy and two members of his family were also found to be infected with Type 80/81 staphylococcus.

Since the entire herd consisted of over 1,000 cows, it was deemed not feasible to test each animal, and, therefore, milk samples were collected at different times from two bulk storage tanks. One tank represented the accumulated milk from one milking of approximately 125 Guernsey cows; the other tank represented the milk from one milking of approximately 650 Holstein cows. Type 80/81 staphylococcus was isolated from two of 22 bulk milk



samples, collected two weeks apart, incriminating the group of 125 Guernsey cows. Two of the three cows originally found infected belonged to this group. Cultures of milk from each animal in the Guernsey herd revealed that five more cows were shedding staphylococcus Type 80/81.

The antibiograms of staphylococcus Type 80/81 isolated from the dairy cattle were similar to those of the human strains—resistance to dihydrostreptomycin, chlortetracycline, oxytetracycline, and tetracycline; variable resistance to penicillin; and susceptible to chloramphenicol, oleandomycin, bacitracin, neomycin, carbomycin, and novobiocin. Theories as to the origin of Type 80/81 infection in human beings and cows are presented, but the actual source of infection could not be determined.

Although associated with mastitis, it appeared that the strains of staphylococcus Type 80/81 observed were similar in pathogenicity and communicability to other coagulase-positive staphylococcus found in dairy cattle. Selected antibiotic therapy in one cow failed to eliminate the organism. Type 80/81 staphylococcus persisted in the milk of this cow for five weeks and in the milk of another for six weeks, at which time the animals were slaughtered.

Bacteriophage Type 52/52A/80, commonly found in human staphylococcal infection on Oahu, was also isolated from the milk of six other cows in the herd.

The public health significance of the findings is discussed.

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