RECOVERY OF *STREPTOCOCCUS AGALACTIAE* FROM A HERD OF LOW PREVALENCE OF INFECTION: A METHOD OF SURVEILLANCE AFTER ELIMINATION OF INFECTION[1, 2, 3]

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(Received for publication April 23, 1969)

Abstract

Three sources of inoculum were examined using TKT medium for detection of *Streptococcus agalactiae* from a herd of low prevalence of infection. An inoculum of a loopful (0.01 ml) from a bulk milk sample yielded a 3.6% recovery rate. An inoculum of gravity cream from bulk tank samples yielded a 36.5% recovery rate. In a third technique, a loopful of rinsings from the filter pad, through which a complete milking had been filtered, yielded a 62.0% recovery rate.

Minett et al. *(3)* demonstrated as early as 1933 that *Streptococcus agalactiae* could be eliminated from individual dairy herds and that herds could be maintained free from infection. Postle *(5)* demonstrated the efficiency of a selective and differential medium (TKT medium) for identifying CAMP test positive streptococci from pooled herd milk. It was shown that more than 5% of quarters must shed S. *agalactiae* in order to permit isolation of these organisms on TKT medium from a single bulk sample.

A method of surveillance after elimination of infection from a herd would be useful in an eradication program. Infection can be reintroduced into the herd from heifers with latent infections *(6)*, by dry cows not examined during the period of elimination *(2)*, or by purchase of infected animals. In the investigations described in this report attempts were made to detect S. *agalactiae* from pooled milk and herd filter pads from a herd of low prevalence of infection.

Materials and Methods

Source of samples. Bulk milk samples and filter pads were collected from an experimental herd in which approximately 40 lactating cows were maintained. After having eliminated S. *agalactiae* from the herd, one quarter of one cow was intentionally reinfected with S. *agalactiae*. This animal was milked last.

Sources of inoculum. Three sources of inoculum for culture were examined. *(a)* A loopful (0.01 ml) of milk from a well mixed 2 oz sample was inoculated onto TKT medium *(5)*. *(b)* Gravity cream *(1)* (12-24 hr standing) from a 2 oz sample was inoculated onto TKT medium with a sterile cotton swab. *(c)* The filter pad that was used during that milking was rinsed with 30 ml of saline solution, and a loopful (0.01 ml) of the rinsing was inoculated onto TKT medium. These methods were examined using samples from 137 milkings.

Table 1. Recovery of streptococcus agalactiae from a herd of low prevalence of infection[1]

Inoculum	Isolation of S. *agalactiae*	
	n	%
0.01 ml loop from 2 oz bulk samples	5	3.6
12-24 hr gravity cream from 2 oz bulk sample	50	36.5
0.01 ml loop from 30 ml saline rinse from herd filter pad	85	62.0

[1]137 trials were performed using each method.

All colonies that were hemolytic on TKT medium were presumed to be S. *agalactiae* or *Streptococcus uberis*.. In order to differentiate S. *agalactiae* from CAMP-test S. *uberis* all colonies hemolytic on TKT medium were transferred to esculin-ferric citrate blood agar *(4)* to determine CAMP test reactions and ability to hydrolyze esculin.

Results

Results comparing the efficiency of three sources of inoculum for identification of S. *agalactiae* from a herd of low prevalence of infection are presented in Table 1. Rate of recovery was highest (62.0%) using the filter pad method, the gravity cream method yielded S. *agalactiae* from 36.5% of the samples, and the recovery rate was lowest (3.6%) when a loopful (0.01 ml) of bulk milk was used.

A probability chart (Table 2) was developed for the gravity cream and filter pad methods of recovery. This shows the number of consecutive negative cultures needed to indicate with any given probability that a herd of 40 cows would be free of S. *agalactiae* infection. For example, with a herd of this size, if

[1]Supported in part by Public Health Service Research Grant No. UI 00124 from the National Center for Urban and Industrial Health.
[2]Published with the approval of the Wisconsin Agricultural Experiment Station as paper N.S. No. 599.
[3]The authors wish to thank Miss Karen Mandt for assistance in the laboratory.

TABLE 2. PROBABILITY TABLE[1]

P	Number of negative tests									
	1	2	3	4	5	6	7	8	9	10
Cream swab	.36	.60	.75	.84	.90	.94	.96	.975	.986	.99
Filter pad	.62	.85	.95	.98	.99					

[1]Note: $\underline{P} = 1 - q^{\underline{n}}$ when

$\underline{P}$ = probability that there is no S. *agalactiae* infection in a herd of 40 cows.

q = percentage of negative tests on 137 trial cultures.

the cream swab method were performed on 10 consecutive samples with negative results, there would be a 99% certainty that none of the animals would be infected with S. *agalactiae*. For comparable assurance with the filter pad method, only five consecutive negative tests would be required.

DISCUSSION

The efficiency of TKT medium for identification of S. *agalactiae* in bulk milk samples from a herd of low prevalence of infection is apparent. Culturing only a single loopful of the bulk milk resulted in too few recoveries (3.6%) to be useful as a screening method in such herds, whereas cream swabs or filter pad rinses as culture inoculum were adequate for this purpose. The filter pad technique was the more sensitive, with a recovery rate of 62%. It should be pointed out that the cow which had S. *agalactiae* infection in one quarter was placed last in the milking order in order to reduce the possibility of spread of the infection throughout the herd. Since this milk was filtered last, it might have favored recovery by the method using filter pads. It would be necessary to repeat the study with random order milking to establish this point.

In an attempt to obtain data from the field for this investigation, several farmers were asked to supply filter pads which had been used in the previous milking. Less than half were willing to cooperate. The reluctance encountered in this initial survey would probably be found to an even greater extent in an official program.

The cream swab method is effective when used repeatedly. It requires no special samples other than a bulk sample. This would provide a surveillance system to detect exacerbation of latent infections or reintroduction of infection in herds from which S. *agalactiae* infection had recently been eliminated. The probability chart (Table 2) gives an indication of the number of samples that must be examined.

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