EFFECTS OF EPHEMERAL FEVER ON MILK PRODUCTION AND REPRODUCTION OF DAIRY CATTLE

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ABSTRACT

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The effect of ephemeral fever (EF) on the lactation, pregnancy and fertility of 19 dairy cows was studied. During the febrile reaction milk yields were reduced by an average of 58,7 ± 22%, depending on the stage of lactation at which the infection was contracted. Ephemeral fever predisposes lactating udders to mastitogenic bacterial infections by deleteriously affecting the leucocytic udder barrier. Delayed oestrus was associated with EF in five out of 14 cows examined.

INTRODUCTION

Ephemeral fever is a cattle disease of tropical and subtropical climates. It was first described in Rhodesia (Bevan, 1907) and has subsequently been reported from South Africa (Theiler, 1908), Kenya (Kennedy, 1915), India (Meadows, 1919), Palestine (Rosen, 1931), Sumatra (Burggraaf, 1932), Australia (Mulhearn, 1937) and Japan (Inaba, Tanaka, Sato, Ito, Omori & Matumoto, 1968).

The clinical symptoms of EF have been recorded in detail by Bevan (1907), Mackerras, Mackerras & Burnet (1940), Mulhearn (1937) and Snowdon (1970). A comprehensive account of the disease is given by Henning (1956) and the pathology has also been described by Basson, Pienaar & Van der Westhuizen (1970).

The effect of EF on dairy cattle has been discussed by Bevan (1907); Mackerras *et al.* (1940); MacFarlane & Haig (1955) and Henning (1956). The gross economic significance of this disease is difficult to assess. Recumbency may lead to severe loss in condition, which may be particularly important when valuable animals are involved (Newton & Wheatley, 1970) and freshened animals may stop nursing their progeny for about two weeks (Kennedy, 1915). Significant economic losses may result from reduced milk production (Morgan & Murray, 1969; Newton & Wheatley, 1970) and from decreased butterfat production and changes in cream quality (Gray, 1938).

The experiment reported below was designed to provide more concrete data concerning the effect of EF on the milk yield, udder health and fertility of dairy cattle.

MATERIALS AND METHODS

1. *EF virus*

The infective virus originated from experimentally infected cattle bled at the peak of the febrile reaction. Blood was collected aseptically in 1% sodium citrate and immediately centrifuged in 250 ml bottles at 2 000 g for 30 minutes using a refrigerated centrifuge. The plasma was removed and discarded without disturbing the sedimented cells. The buffy coat (BC) was aspirated with a syringe and diluted with phosphate buffer to give a 10% suspension. Some admixture of erythrocytes with the BC suspension was inevitable. Aliquots (5 ml) of the BC suspension were stored in sealed ampoules at −70°C in a dry ice cabinet until required, when one was injected intravenously into each experimental animal.

2. *Experimental animals*

Nineteen EF susceptible cows belonging to the Institute's dairy herd were used. Details of the breed, stage of lactation, pregnancy and serum neutralizing antibody indices prior and subsequent to EF infection are summarized in Table I.

3. *Examination of experimental animals*

Monthly udder health records prior to the experiment were available for each cow and milk samples were examined on at least two successive days immediately before the artificial infection to obtain control values. Daily udder and milk examinations were continued for at least seven days after the disappearance of clinical symptoms. The presence of mastitis was determined according to international diagnostic standards (Kästli, 1967) and techniques described by Giesecke, Van den Heever, Hope & Van Staden, 1968; Giesecke, Nel & Van den Heever, 1968. In milk samples from cows 6965, 3054, 7638 and 5282 cells were differentiated into epithelials and leucocytes because these animals had not experienced any mastitis. In the other cows only leucocytic cells were counted.

Rectal temperatures were taken prior to inoculation and subsequently at four-hourly intervals for ten days and nights. The cows were subjected to daily clinical examinations during this period. Their calves were kept separately and bucket-fed.

The stage of pregnancy and condition of the ovaries and genital tracts were determined by rectal examination performed prior and subsequent to experimental infection with EF virus and were also based on records of the dates of artificial inseminations, oestrus and calving.

4. *Neutralization tests*

Blood samples collected from each experimental animal before and three weeks after artificial infection were allowed to clot at 4°C. The samples were centrifuged at 600 g for 30 minutes, the serum decanted into sterile bottles and stored at 4°C. A 10% mouse brain suspension of the mouse-adapted prototype EF 1 virus in phosphate buffer was used as antigen. The material was stored in 0,5 ml aliquots in sealed ampoules at −70°C in a dry ice cabinet (Van der Westhuizen, 1967). In the test, equal volumes of antigen dilutions (10^{-1} to 10^{-7}) and undiluted serum were mixed and incubated in a water bath at 37°C for 45 minutes. One family of seven suckling albino mice
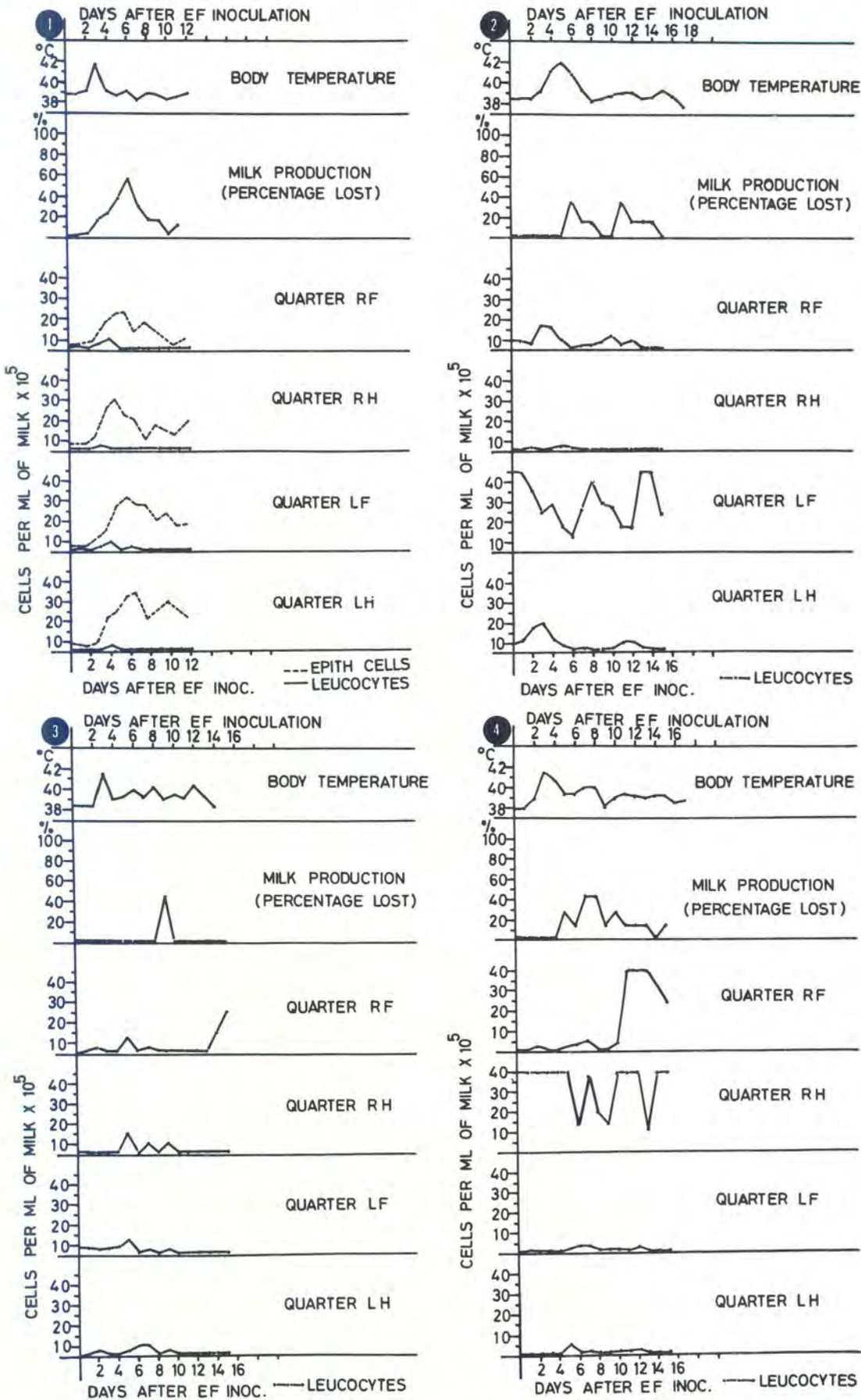
FIG. 1 to 4 Body temperature, milk production and milk cytology of Cows 6965, 8601, 8596 or 8595 respectively

TABLE 1 Details of experimental animals used

Cow number	Breed	Stage of lactation	Stage of pregnancy*	Serum neutralizing index**	
				before infection	3 weeks after infection
6965	Friesian	35 days	Just calved	<0,5	>5,5
8601	Jersey	3 days	Just calved	<0,5	5,0
8596	Jersey	1 day	Just calved	<0,5	5,0
8595	Jersey	5 days	Just calved	<0,5	5,0
3054	Friesian	3,5 months	1,5 months	<0,5	>5,5
8555	Jersey	3,5 months	No data	<0,5	>5,5
8569	Jersey	4 months	1,5 months	1,1	>5,5
7638	Brown Swiss	4 months	No data	<0,5	>5,5
6954	Friesian	6 months	No data	0,7	>5,5
5282	Friesian	6,5 months	3 days	<0,5	>5,5
5634	Friesian	7 months	No data	<0,5	>5,5
2830	Friesian	8 months	4 days	0,9	>5,5
7550	Friesian	8,5 months	2,5 months	<0,5	>5,5
8567	Jersey	No data	2 months	0,8	>5,5
8557	Jersey	No data	4 weeks	0,8	>5,5
8607	Jersey	No data	5 weeks	0,6	>5,5
8570	Jersey	No data	5 months	0,8	>5,5
8609	Jersey	No data	5 months	<0,5	>5,5
8559	Jersey	No data	8 months	0,9	>5,5

*The stage of pregnancy was calculated from the last date of artificial insemination, subsequent lack of oestrus, rectal examination and date of calving.
**Neutralization index expressed as log 10 mouse LD_{50} neutralized.

was inoculated intracerebrally with 0,03 ml each of a serum-virus mixture and observed for seven days during which any mortalities were recorded. Known negative and positive EF sera were included as controls. The 50 % end points were calculated according to the method of Reed & Muench (1938) and the neutralization indices were expressed as the log 10 mouse LD_{50} virus neutralized.

RESULTS

1. *The effect of EF on the udder of cows during the early stages of lactation*

The animals concerned (6965, 8601, 8596 and 8595) had calving dates ranging from 1 to 35 days prior to artificial infection (Table 1).

All four developed distinct febrile reactions between the 3rd and 4th day after inoculation, followed by characteristic EF symptoms such as fever, enlargement of superficial lymph nodes, excessive salivation, general stiffness and nasal discharge. Their temperatures, milk production and milk cytology are recorded in Figures 1 to 4.

Cow 6965 only developed mild symptoms of EF but showed a marked temporary drop in milk production (60%) concomitant with the rise in temperature. Milk production returned to pre-trial values as soon as the fever ceased. Somatic cell counts in the milk, mainly epithelial cells, increased distinctly during the febrile reaction, but gradually returned to pre-trial values thereafter. At the peak of the febrile reaction *Staphylococcus epidermidis*, *Streptococcus dysgalactiae* and *Pseudomonas aeruginosa* were also isolated repeatedly subsequent to the febrile reaction but clinical symptoms of mastitis were absent. According to international standards on somatic cell counts, Cow 6965 showed subclinical mastitis in all four quarters on the 3rd day after inoculation and this persisted for at least 12 days after inoculation, when the examination was discontinued.

Cow 8601 did not develop severe stiffness but during the febrile episode milk yields were reduced by 33%. Leucocyte counts were erratic though they tended to decrease, particularly in the two hind-

quarters. *Streptococcus dysgalactiae* was constantly isolated from quarter LF and this, together with the leucocyte counts, suggests that subclinical mastitis was present in this quarter before EF inoculation. *S. aureus* was repeatedly isolated from quarter RF and this plus the leucocyte counts suggest that subclinical mastitis developed on the day following EF inoculation and persisted throughout the remaining 15-day observation period.

Cow 8596 developed severe stiffness in all four legs and lameness of the right hind leg, then became recumbent on the 7th day after inoculation. She was unable to stand for three days, but stood up on the 4th day with assistance. Milk production dropped by 42% when she was recumbent but returned to pre-trial values as soon as she was able to stand again. Leucocyte counts in the four quarters averaged $2,3 \times 10^6$ cells prior to infection, decreased sharply on EF inoculation, returned to distinct peak values between the 5th and 9th day after inoculation and then returned to low levels on the 10th day. Subsequently one quarter (RF) became infected with *S. aureus*.

Cow 8595 showed a diphasic febrile reaction and was lame in the front legs. Milk production decreased by 43% during the primary and 13% during the secondary febrile response. Milk yields returned to pre-trial values on the 13th day after inoculation. Leucocyte counts of quarters RF, LF and LH were very low, showing peaks during the febrile reaction comparable to those seen in cow 8596. On the 10th day after EF inoculation quarter RF showed subclinical mastitis due to infection by *S. aureus*. Quarter RH showed subclinical mastitis due to *S. epidermidis* prior to EF inoculation.

2. *The effect of EF on the udder of cows in the middle stages of lactation*

The cows concerned (3054, 8555, 8569, 7638 and 6954) had calving dates ranging from 3,5 to 6 months prior to artificial infection (Table I).

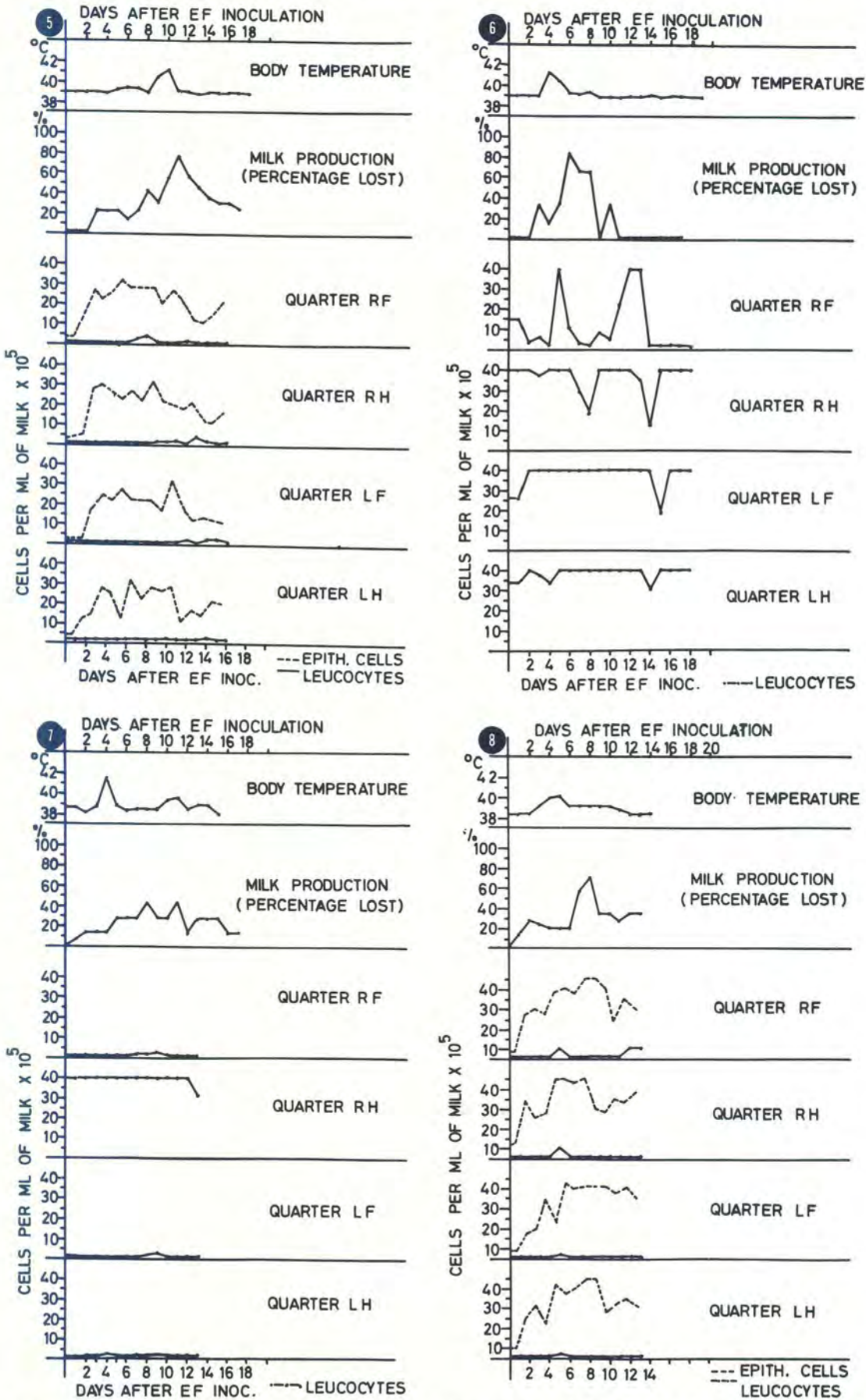Cows 3054 and 8555 were at the start of mid-lactation and both developed symptoms of EF. During

FIG. 5 to 8 Body temperature, milk production and milk cytology of Cows 3054, 8555, 8569, or 7638 respectively

pyrexia their milk production fell by 70% and 90% respectively (Fig. 5, 6). When the temperature of Cow 8555 dropped her milk yield returned to normal on the 12th day but in Cow 3054 it only improved gradually and had not yet reached the pre-trial level on the 17th day after EF inoculation, when examinations were discontinued.

Throughout the entire period of examination Cow 3054 showed low leucocyte counts. According to international standards on somatic cell counts, she showed subclinical mastitis two days after EF inoculation, mainly due to an increase in the epithelial cell fraction. Although epithelial counts improved gradually, subclinical mastitis was still apparent at the end of the experiment, 17 days after EF inoculation.

Cow 8555 had a chronic clinical S. aureus mastitis in quarters RH, LF and LH. Quarter RF was affected by subclinical aseptic mastitis and leucocyte counts reached peak values on the 4th and 11th days after EF inoculation, the first increase coinciding with the isolation of S. aureus from the milk samples. In the other quarters (RH, LF and LH), from which S. aureus was isolated daily, a decrease in leucocyte counts occurred on the 2nd, 3rd and 5th days and between the 12th and 14th days after EF inoculation.

Cow 8569 (Fig. 7) was in the 4th month of lactation and developed a slight febrile reaction only. Milk production decreased during the febrile reaction by about 40% and, though it subsequently improved it never returned to the pre-trial level during that lactation. No significant changes in cell counts were observed in the healthy quarters RF, LF and LH or the mastitic quarter RH during the period of examination. Quarter RH was a long standing case of S. aureus mastitis.

Cow 7638 (Fig. 8) was in the 4th month of lactation and following the febrile reaction her milk yield was reduced by about 70%. Subsequently her production improved considerably but it remained 30% below the pre-trial level for the remainder of the lactation. Leucocyte counts showed slight increases between the 4th and 6th days after EF inoculation. Epithelial cell counts increased considerably during the EF reaction and remained at high levels thereafter. According to international standards on somatic cell counts, this cow showed subclinical mastitis in all four quarters following EF inoculation.

Cow 6954 (Fig. 9) was in the 6th month of lactation and exhibited a 95% reduction of milk yield during the EF reaction. Production returned to the pre-trial level a few days after her temperature returned to normal. Leucocyte counts of all four quarters increased on the 4th day after EF inoculation. Then returned to pre-trial values two days later. They remained low for the subsequent four days but increased again on the final day of examination, during which Pseudomonas aeruginosa was isolated in large numbers from all four quarters. No clinical symptoms of mastitis were evident.

3. The effect of EF on the udder of cows in the advanced stages of lactation

The cows were numbers 5282, 5634, 2830 and 7550 with calving dates ranging from 6,5 to 8,5 months prior to artificial infection (Table 1).

Cow 5282 (Fig. 10) was 6,5 months in lactation and reacted severely. She went down on the 4th day after infection and remained recumbent for five days, during which she made no attempt to stand up, either on her own or when assisted. On the 10th day after infection she stood up with assistance. Milk production dropped rapidly during the febrile reaction and was 55% less than the normal yield on the 8th day after inoculation after which milking was discontinued until she could stand again. Her milk yield subsequently was very low and did not return to pre-trial levels for the duration of the lactation. During the febrile reaction there was a rapid rise in epithelial cell counts, which remained extremely high throughout the entire period of examination. Leucocyte counts increased erratically. S. aureus was isolated from all four quarters on the 2nd day after EF inoculation but subsequent milk samples from quarters RF, RH and LH were negative. It was repeatedly isolated from quarter LF but, as there was a pre-trial history indicating occasional brief infections with S. aureus in this quarter, these isolations are unlikely to have been associated with the EF reaction.

Cow 5634 (Fig. 11) was seven months in lactation. She showed severe clinical symptoms, including recumbency for several days, although she could stand up with assistance. Milk production decreased by about 40% but had returned to pre-trial levels 14 days after the febrile reaction had ceased. Leucocyte counts increased slightly on the 4th and 7th days and exhibited distinct peaks between the 8th and 11th days after EF inoculation. S. aureus was isolated repeatedly from all four quarters but could not be correlated with the increase in leucocyte counts.

Cow 2830 (Fig. 12) was eight months in lactation. Her reaction was similar to that of Cow 5634 as far as general symptoms were concerned. However, her milk production was affected more severely, since she gave 90% less milk between the 3rd and 7th days after EF inoculation. On the 7th day her milk production returned to 50% of the pre-trial level and it remained low for the duration of the lactation. Leucocytic counts revealed different reactions for the individual quarters. Quarter RF was mastitis negative and showed a sharp increase of leucocytes between the 5th and 11th days, but returned to pre-trial levels on the 12th day after inoculation of EF virus. Quarters RH and LH had a history of chronic clinical S. aureus mastitis dating back for several months. Leucocyte counts in both these quarters were equivalent to or higher than 4×10^6 cells/ml of milk for three days after EF inoculation, then became very much reduced between the 4th and 7th days and subsequently returned to pre-trial levels. Quarter LF had no previous history of mastitis but became infected on the 6th day after EF inoculation and thereafter S. aureus was isolated from it daily. The onset of infection in this quarter coincided with increased leucocyte counts. These decreased again between the 10th and 13th days, but reached a new peak on the 14th day without evidence of clinical mastitis.

Cow 7550 (Fig. 13) was in lactation for $8\frac{1}{2}$ months and developed symptoms of EF similar to those of Cows 5634 and 2830. Milk production decreased by 50% during the febrile reaction but returned to pre-trial levels immediately after the fever had subsided. However, milk yields fluctuated considerably between the 10th and 18th days before normal yields were re-established. Leucocyte counts for the individual quarters varied. Quarter RF showed an increase between the 5th and 9th days after EF inoculation and fluctuated at low levels thereafter. The leucocyte reaction in quarter RH was basically similar to that of
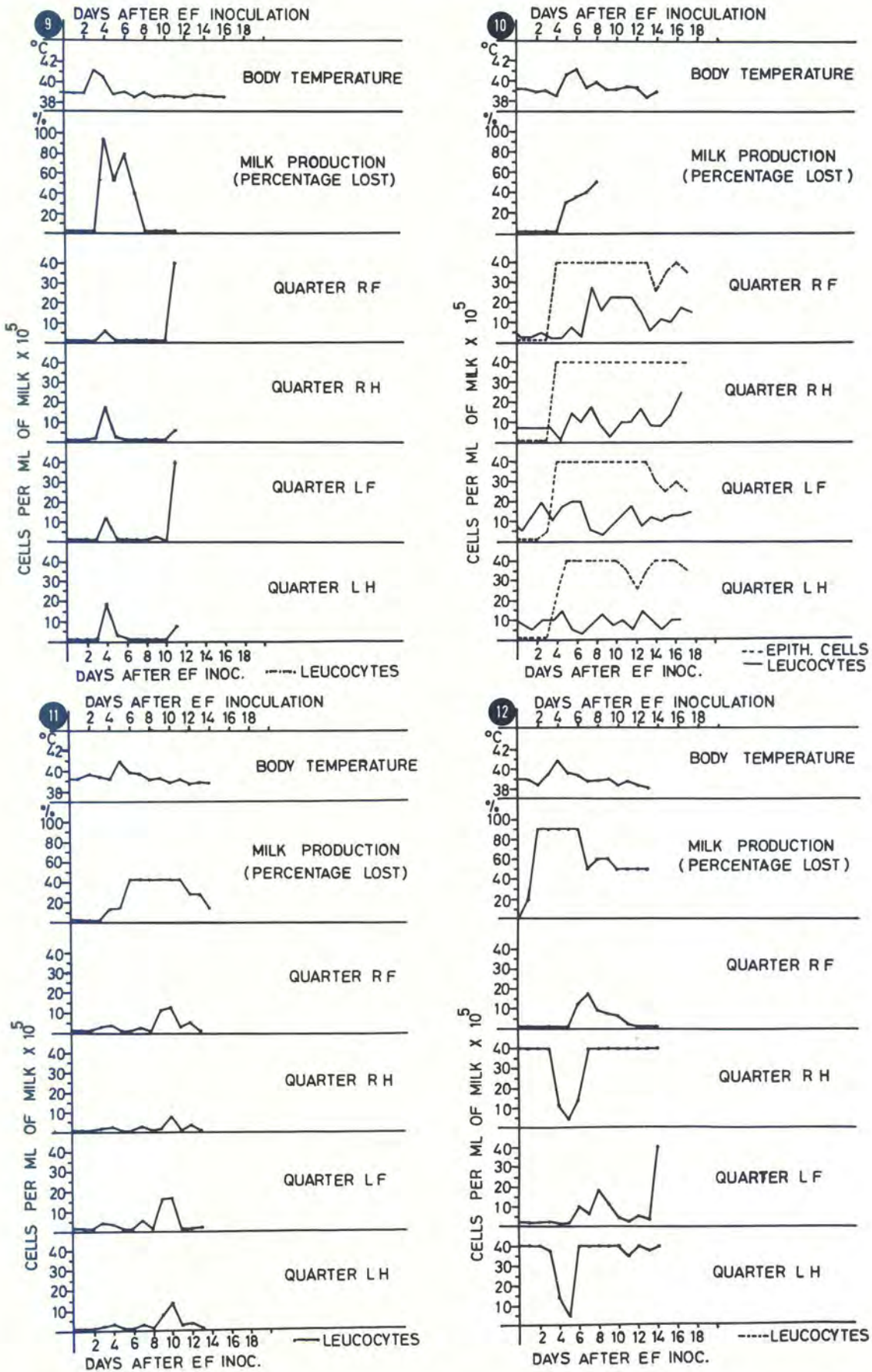
Fig. 9 to 12 Body temperature, milk production and milk cytology of Cows 6954, 5282, 5634 or 2830 respectively

RF but reached higher cell numbers and produced two distinct peaks on the 8th and 13th days after EF inoculation. For quarter LH the leucocyte counts were even higher than those for quarter RH, and more erratic but they were in general similar. Quarter LF contracted subclinical *S. aureus* mastitis on the 2nd day after EF inoculation and the udder infection persisted throughout the period of examination.

4. *The effect of EF on pregnancy and fertility*

With the exception of Cows 8555, 7638, 6965, 6954 and 5634, for which pregnancy data were not available, all the animals listed in Table 2 were observed to see what effect EF would have on pregnancy and fertility.

These observations were only made on a few animals, so cannot be regarded as conclusive, but they do give an indication of the effects of this virus. All the animals reacted to EF inoculation. Two cows, inseminated four and five weeks respectively before the EF inoculation, did not calve and remained in anoestrus for prolonged periods. They either did not conceive or the foetuses were resorbed or discarded unnoticed. Three cows that were inoculated with EF one to five days after calving failed to come into oestrus for six months or more (Table 2).

5. *Other complications*

Four of the 19 animals under observation developed ocular lesions during the febrile reaction. Although the corneae of two animals were clear they could not see properly. The corneae of both eyes of the two other animals gradually became whitish and opaque. Macroscopically these ocular changes were not associated with symptoms of inflammation. This corneal opacity cleared up within some 10 to 14 days and the animals seemed to have regained undisturbed vision thereafter.

DISCUSSION

The evidence obtained indicates that the following effects of EF cause major losses in the cattle industry, especially to dairy farmers:
(a) decreased milk yields,
(b) incorrect positive mastitis diagnosis,
(c) predisposition of udders to bacteriogenic mastitis and
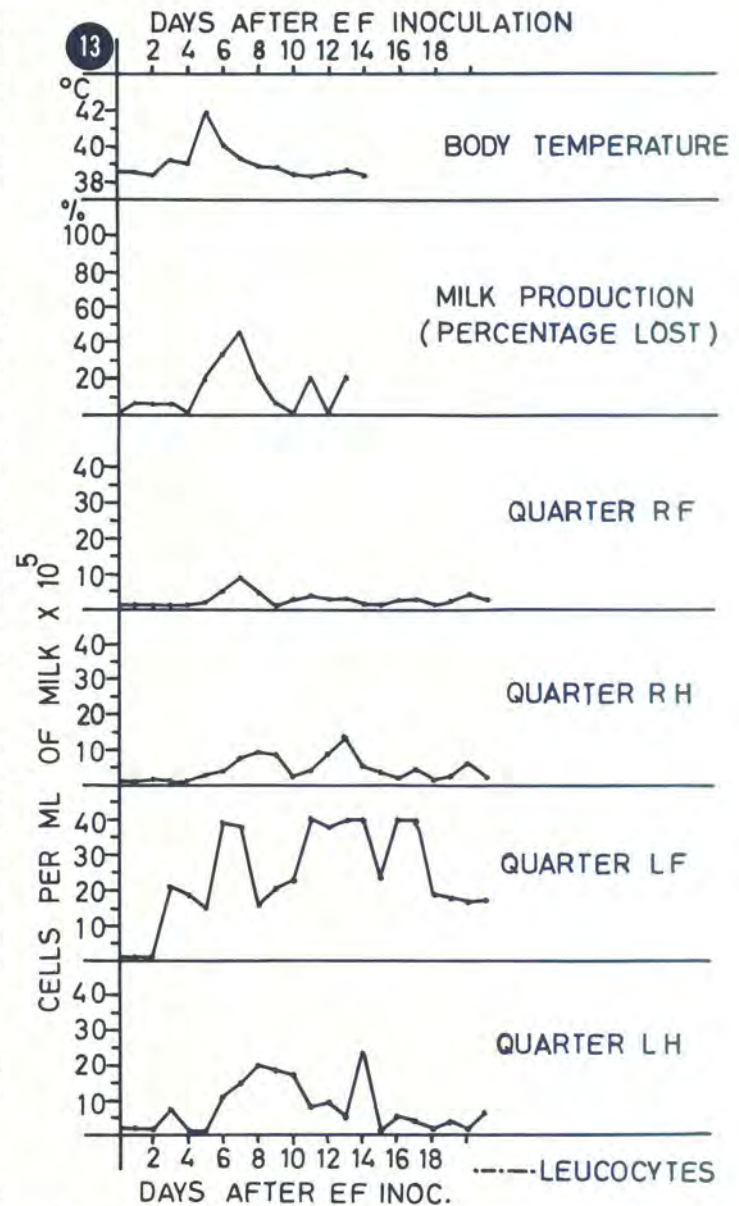(d) loss of condition or death of affected animals.

FIG. 13 Body temperature, milk production and milk cytology of Cow 7550

TABLE 2 Observations on pregnancy and fertility of cows inoculated with virus

Cow no.	Breed	Stage of pregnancy	Reaction to EF	EF and concomitant pregnancy	EF and subsequent fertility
5282	Friesian	3 days**	5/2 T 41,1°C*	Calved normally	Not examined
8567	Jersey	2 months	4/2 41°C	Aborted during febrile reaction	Not examined
3054	Friesian	1,5 months	6/1 T 39,5°C 9/2 T 41,1°C	Calved normally	Not examined
7550	Friesian	2,5 months	4/2 T 41,7°C	Calved normally	Not examined
8609	Jersey	5 months	3/1 T 40,7°C 8/6 T 40,5°C	Calved normally	Not examined
8559	Jersey	8 months	7/2 T 39,9°C	Calved normally	Not examined
8570	Jersey	5 months	6/1 T 40°C	Weak calf died on delivery	Not examined
2830	Friesian	4 days**	3/3 T 40,7°C	Calved normally	Not examined
8601	Jersey	3 days p.p.***	4/3 T 41,9°C	—	Anoestrus for 6 months
8596	Jersey	1 day p.p.	3/4 T 41,4°C	—	Anoestrus for 6,5 months
8595	Jersey	5 days p.p.	3/4 T 41,4°C	—	Anoestrus for 8 months
8557	Jersey	4 weeks**	4/3 T 41,5°C	Did not calve	Anoestrus for 7 months
8607	Jersey	5 weeks**	10/1 T 40,5°C	Did not calve	Anoestrus for 11 months
8569	Jersey	1,5 months	4/1 T 41,6°C	Calved normally	Anoestrus for 6 months

*5/2 T 41,1°C = Animal reacted on the 5th day after artificial infection with EF virus; the reaction lasted two days and the highest rectal temperature (T) recorded was 41,1 C.
**Based on dates of last oestrus, insemination and subsequent delivery.
***p.p. = days *post partum*
These observations show that six cows infected with EF virus when pregnant for periods varying from 3 days to 8 months calved normally. One cow aborted during the febrile reaction and another gave birth to a weak calf which died on delivery.

The average daily loss of milk production occurring in early, middle and late lactation was 44,5 ± 13%, 73,0 ± 27% and 58,7 ± 25% respectively during the acute EF reaction. The total losses caused by EF virus are even greater than these figures suggest because cows in different stages of lactation seem to have different potentials for the recovery of their milk production. The production of cows infected during early lactation seems to return to pre-trial levels more readily than that of cows infected later, apparently because there is a tendency towards proliferative cellular activities during early lactation and involutive cellular activities during the later stages. Thus, cellular proliferation of udder epithelia in the early stages of lactation apparently compensates very quickly for any epithelial losses caused by the febrile reaction and milk production returns to pre-infection levels shortly after the fever has ceased. During the later stages, though, a distinct involutionary tendency of udder epithelia appears to be enhanced by the febrile reaction and the milk production of these cows seldom, if ever, returns to pre-infection levels. However, they should produce normal milk yields after calving again.

Even if the milk production of cows that have been infected with EF does return to normal there is a strong possibility that this milk will be classified as being unfit for human consumption because its somatic cell content is considered too high. Present international standards suggest that milk should be considered abnormal if the somatic cell content exceeds 500×10^3 cells/ml milk, whatever the origin of these cells (Kästli, 1967). Cows producing such milk would probably be treated for mastitis, especially if a contaminant had caused a wrong positive bacteriological result, and their milk would be condemned.

It is extremely doubtful, though, whether a mastitis test based on total somatic cell counts is valid. In cows that have been infected with EF this cell count automatically rises because there is an increase in the number of epithelial cells sloughed off from the basal membrane of the udder epithelium, i.e. involution in the udder, which is a purely physiological phenomenon, increases. Under these conditions it is probably an emergency reaction to prevent the drainage of energy via the udder. The rise in the number of leucocytic cells in the milk, although the cows are mastitis-negative, presumably results from increases in the number of mononuclear cells. The polymorphonuclear neutrophilic cells actually decrease in numbers, apparently because they are utilized increasingly by cows infected with EF, judging by the differential blood counts carried out by Mackerras et al., 1940. This depletion of the polymorph neutrophilic cells explains the reactions seen in mastitic quarters and the occurrence of bacteriogenic udder infections in nine formerly mastitis-negative quarters in our experiments. (The significance of the leucocyte barrier in the bovine udder is discussed in detail by Schalm, Carroll & Jain, 1971).

From the data obtained on the effect of EF on the pregnancy or fertility of the dairy cows it is obvious that the delays in oestrus seen in five out of 14 animals (of which one has not been in oestrus for eight months and the other for 11 months) would also cause considerable losses to cattle farmers. These losses may be aggravated either by abortions occurring during the earlier stages of pregnancy or by the birth of weak calves after an apparently normal pregnancy.

It would be interesting to know what effect EF virus infections have on the semen quality of bulls, as this might also have a bearing on the reproductive cycle of the cows.

Unfortunately the ocular changes observed in four Jersey cows were not investigated in detail. However, there appear to be two possible causes for these changes: the corneal opacity either could result from lymphocytic infiltration or from metabolic disturbances. Since no inflammatory symptoms were observed macroscopically in the corneae and since there was no purulent exudate from the eyes (as in the joints of EF-infected cows examined by Basson, Pienaar & Van der Westhuizen, 1970) metabolic disturbances seem to be the more likely cause. In this connection, changes in the activity of hyaluronidase (Ham, 1969) could be of particular importance, not only with regard to the ocular alterations per se but even more so for the typical symptomatology of EF-infections in general.

SUMMARY

The clinical symptoms of EF reported by earlier workers were confirmed and elaborated by observations made on 19 dairy cows artificially infected with EF virus.

Information obtained from 13 lactating cows indicate that milk losses caused by EF virus are dependent upon the stage of lactation at the time of infection and on the standards used for the diagnosis of mastitis. Clinical mastitis was not elicited by EF but subclinical bacteriogenic mastitis developed in nine out of the 42 quarters observed during the course of the EF reaction.

In addition milk losses and lowered resistance of the udder, predisposing it towards bacteriogenic mastitis, further economic losses may be caused by factors such as delayed oestrous activity, abortion, delivery of weak calves or other clinical complications.

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