THE EFFECTS OF ANTE PARTUM LEPTOSPIRA POMONA INFECTION ON BOVINE FETAL AND MATERNAL BLOOD VALUES

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by

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A Thesis

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INTRODUCTION

Naturally acquired bovine leptospirosis may manifest all stages of severity from unapparent infection, detectable only by laboratory means, to an acute febrile form leading to death in a few days. Age, breed of cattle, individual resistance, and the particular infecting strain are some of the factors responsible for this great variability.

Young animals are more severely affected, with reports of losses as high as 50% in feeder lot situations (39). Older animals, even with no history of previous exposure to the organism, exhibit fewer acute manifestations, perhaps due to a former unapparent infection or a physiologically acquired resistance. Ferguson, et al. (20), in a limited study, suggested that Jersey cattle may be more susceptible than Holstein-Friesian, and this might be extended to other breeds. Leptospira pomona is the usual infecting serotype in the United States, but even in this supposedly serologically homogeneous species, certain strains are more virulent than others. L. sejroe, L. hyos, L. grippotyphosa, L. canicola, L. icterohemorrhagiae, and the recently isolated L. hardjo have been implicated by serological procedures (21).

Some variation is found in the reports of the severity of the disease in pregnant cattle. Abortion in late pregnancy is given as the only symptom in most cases (2, 22); while the more severe acute febrile form of the disease has

been described in other papers (39). There is a similar variation in reporting abortion rates. The figure of 5-10% is most generally found, even in herds showing 100% serologically positive animals. However, an occasional abortion "storm" may produce fetal losses as high as 20-40% in large beef cattle herds.

This degree of variation, its many possible causes, and the actual mechanism of the leptospiral abortion has been the subject of much study and debate in recent years. De Punga and Bishop (40) have suggested three major possible causes:

1. Pyrexia and systemic reaction in the maternal organism, resulting in abortion.
2. Localized lesions in the placentomes (maternal-fetal cotyledonary junction) with interrupted transfer of metabolites and subsequent fetal death and expulsion.
3. Actual invasion of the fetus by leptospirae, resulting in acute fetal infection, death, and expulsion.

Several of these postulates have been the subject of further investigation. Morse and McNutt (31) modify the second contention to state that interference with placental and fetal circulation may be the cause or contributing factor in leptospiral abortion. Morter, et al. (34), suggest that hormonal imbalance due to alterations of hormone-producing maternal crypts might be a factor.

Ferguson, et al. (20) propose a soluble toxin, produced by the leptospira in the dam, capable of passing the placental barrier and destroying the fetal erythrocytes, thereby producing anoxia, death, and fetal expulsion.

Fennestad and Borg-Peterson (13, 14, 15) support postulate number three that leptospiral abortion is most often due to fatal leptospirosis of the fetus, and have demonstrated leptospirae in aborted fetal material by silver impregnation methods. They feel that autolysis of the fetus destroys these organisms and accounts for negative results reported in routine culture procedures. Further, in more recent papers (16, 17, 18), they state that acceptable evidence has not been presented to substantiate the toxin hypothesis.

Since erythrocyte destruction can be determined by simple and reasonably accurate methods, it seemed of value to explore Ferguson's proposal as to the action of a hemolytic toxin and determine if fetal blood values were affected by maternal leptospirosis. The hematological constituents chosen for determination were total erythrocyte and leukocyte counts, differential leukocyte determinations, hemoglobin and hematocrit values.

In addition, a selected group of chemical determinations were chosen to help clarify some of the issues advanced in the possible disturbance of maternal-fetal transfer. The blood gases, carbon dioxide and oxygen were naturally concerned in the erythrocyte destruction hypothesis; non-protein nitrogen and creatinine would indicate renal damage, the most consistent feature of leptospirosis; and glucose is a readily diffusable metabolite, reduction of which could severely affect fetal development.

LITERATURE REVIEW

The published reports on fetal bovine blood values are exceedingly sparse. In his comprehensive compilation of blood values in 1953, Albritton (1) found only one reference to fetal cattle. This report by von Deseo (40), in 1929, dealt entirely with the erythrocytic series of blood cells, giving values for red blood cells, hemoglobin and packed cell volume. His main concern was with the relative proportion of total solids and fluid content of the erythrocyte at various stages of fetal life.

Barcroft (4) combined in one volume his own extensive work on ovine prenatal development and all material available on other mammalian species. This included one short paragraph on oxygen dissociation curves in a single eight month old bovine fetus. The original article by Roos and Romijn (37), however, is more detailed and reviews the work on oxygen capacity in several species. In man, the maternal oxygen capacity was higher than the fetal, but in rabbits, dogs, sheep, goats and guinea pigs, the fetal values were 15-25% higher than the maternal. These authors used oxen to establish normal values in non-pregnant cows at 10.28-18.45 volumes per cent with an average of 14.55. Determinations were also made on oxen in the seventh to ninth month of pregnancy, and eight 7½ to 8½ month fetuses. Fetal blood in the last month of uterine life has a 2-12% higher oxygen capacity than the maternal blood and the difference becomes

smaller as term is approached. The oxygen dissociation curves mentioned in Barcroft's book show a large gap between fetal and maternal curves proving, according to the authors, the great oxygen avidity of the fetal blood. The great disparity between maternal and fetal carbon dioxide curves led them to theorize the now proven difference between maternal and fetal hemoglobins.

Further search of the literature failed to reveal any recent publications dealing specifically with bovine fetal blood values. The economic aspect would appear to be a limiting factor and, in fact, Roos and Romijn admit that the expense prevented further experimentation.

Voluminous reports have appeared on various aspects of fetal life in other mammalian species, particularly the sheep. Many of these publications entitled "fetal studies" are in reality on newborn animals with the blood samples being obtained from the umbilical cord at birth (12, 25, 27). It is impossible to completely extrapolate work from one species to another, but the sheep and cow are fairly close physiologically, and some of the findings could be used as guideposts, at least. A volume by Barclay, Franklin and Pritchard (3) on fetal circulation (1945) reviewing the work to date contains many comparative studies on many mammals, but the sheep is still the primary example, with the human fetus second in volume of experimental work.

The group of Dawes, Mott and Widdicombe in various combinations (9, 10), has extensive current publications on fetal circulation and various blood gas relationships in

fetal lambs. The work of Metcalfe, et al. (30), on uterine blood flow and oxygen comsumption, compares oxygen diffusion in three species of animals with different types of placentation. Following Barron's (5) hypothesis that the gradient varies with the thickness of placental membrane, they found that the values for the three layered human placenta fall between those for the single layered rabbit and the five layered sheep.

Likewise, certain human studies may have implications, in a general way, for the values found in this study. Turnbull and Walker (41), studying red blood cell and hemoglobin concentrations in human fetal bloods, found that the hemoglobin values stabilize at 15 grams during the last trimester of pregnancy, while the number of erythrocytes and the packed cell volume increases as pregnancy advances. This suggests that the red cell becomes progressively smaller as fetal age increases. In fact, in a sequel to this article, Walker and Turnbull (43) state that any red cell over nine micra in diameter is a fetal cell and that adult and fetal hemoglobin is contained in separate cells.

The normal adult bovine values are found in such standard physiology texts as Dukes (11) and Coffin (8). Ferguson, et al. (19), report no significant relationship between progression of pregnancy with changes in numbers of red and white cells per unit volume or the percentage of different types of white cells. Reineke, et al. (36), report a series of carbon dioxide:oxygen ratios and respiratory quotients on

goat arterial and mammary gland blood which might likewise be an indication in bovine blood gas relationships. Unfortunately, Shaw's extension of this work to cattle gives only the differences in volumes per cent rather than the values themselves (38). These would have been useful criteria in the maternal studies on oxygen and carbon dioxide.

MATERIALS AND METHODS

Thirteen apparently normal pure bred Holstein-Friesian heifers 24 to 30 months old were used in this project as experimental animals. They had been obtained at 8 to 12 months of age and kept free from contact with other animals. All were official Brucella abortus strain 19 vaccinates, and their sera were negative for leptospiral antibodies as determined by the modified agglutination lysis test (32) with L. pomona (Johnson), L. canicola, and L. icterohemorrhagiae (AB) antigens. The heifers were bred artificially with semen kindly supplied by the Michigan Artificial Breeders Cooperative.

The carotid arteries were exteriorized in order to obtain the maternal blood samples more easily. From one to six pre-infection determinations were made on each animal to establish base normals.

At stages varying from the fourth to the eighth month of pregnancy, seven of the heifers were inoculated subcutaneously with five ml of L. pomona (strain Wickard). The inoculum was heparinized guinea pig or hamster blood obtained during leptospiremia. This strain of L. pomona was chosen for its virulent properties and had been kept in animal passage since its isolation from the urine of an infected cow. Controls received 5 ml of normal blood from guinea pigs or hamsters. One animal was intra-uterinely infected as described by Morter (33). The one remaining animal was found

to be not pregnant, so was designated as a normal, non-pregnant, non-infected control. See Table 1 for animal numbers, period of gestation, route of inoculation and final disposition.

Bacteriological and serological evidence of infection has been reported elsewhere (33), since these animals were used for several different aspects of experimental leptospirosis.

The day of necropsy, a maternal arterial sample was obtained, then a hysterotomy was performed after tranquilization with Diquel® and anaesthetization by a procaine T block of the left paralumbar fossa. Fetal venous and arterial samples were obtained from the umbilical vein and artery for comparative studies, especially of the blood gases. Due to the large number of separate determinations desired, it was necessary to procure at least 20 ml of blood from each site and divide this into 4 separate samples. The method of treatment of these samples, the determinations for which each was employed, and the test procedure used in obtaining values are as follows:

1. 5 ml of blood added to dried 20% potassium oxalate under a layer of mineral oil, for the blood gas determinations using the Van Slyke and Neill method for simultaneous determination of carbon dioxide and oxygen in a single sample (35).

2. Approximately 3 ml of blood combined with 0.1 ml of 10% potassium oxalate for most of the remaining chemical and hematological determinations. Total erythrocyte and

leukocyte values were calculated by standard methods, and the new cyanmethemoglobin procedure was used for the hemoglobin (7). For efficiency, the three remaining chemical values were all determined on Folin-Wu filtrates; glucose by the method of Folin and Wu; non-protein nitrogen by the Koch and McMeekin modification of the original Folin and Wu method; and the creatinine by the Folin and Wu alkaline picrate method (24).

3. 2-3 ml of blood added to the dried residue of 0.5 ml of a combination potassium and ammonium oxalate for the hematocrit estimation after the method of Wintrobe (7).

4. Two blood smears were made on glass slides, stained by Wright's method, and examined for the differential leukocyte determination (5).

RESULTS

The hematological and chemical values obtained in the various determinations on maternal and fetal blood at various stages of gestation are summarized in Tables 2 through 6. The maternal values for both non-infected and intraperitoneally infected animals compare well with the non-pregnant, non-infected control heifer, and are well within the normal limits as given in Dukes (11) and Coffin (8). There is no basis for comparison of the fetal values, other than the erythrocytic series, and the values given by von Deseo are closely paralleled.

Chart 1 compares the changes in the fetal erythrocytic series produced by the two routes of infection. Figures 1 and 2 depict these changes photographically. The other values for the fetus from the intra-uterinely infected dam were not outside the range established on the remaining fetal samples.

Table 7 gives the respiratory quotients of the fetuses from the 6 month infected, 7 month infected and 9 month non-infected heifers.

Table 1. Experimental Animal Statistics.

Animal Number	Stage of Gestation at Termination in months	Route of Infection
7	6	Intraperitoneal
8	7	Intraperitoneal
10	7	Intraperitoneal
83	7	Intraperitoneal
1	8	Intraperitoneal
5	8	Intraperitoneal
03	8	Intraperitoneal
13	7	Intra-uterine
17	6	Non-infected
3	8	Non-infected
9	9	Non-infected
07*		
4*		

* Normal Controls

Table 2. Erythrocytic Determinations.

Stage of gestation in months	Sample Source	Non-Infected			Intraperitoneal Infection			Intra-Uterine Infection		
		RBC[a]	Hgb[b]	Hematocrit[c]	RBC[a]	Hgb[b]	Hematocrit[c]	RBC[a]	Hgb[b]	Hematocrit[c]
6	P	(1)			(1) 7.29	11.5	37			
	M	7.03	10.8	34	7.82	11.6	37			
	FA	5.96	8.8	36	7.29	7.5	30			
	FV	5.62	8.3	34	6.77	7.6	30			
7	P				(3) 8.70	11.8	39.5	(1) 5.95	10.4	34
	M				8.21	10.2	34.6	2.31	5.1	18
	FA				5.60	7.8	31.0	1.85	3.4	12
	FV				5.68	8.0	32.0			
8	P	(1) 7.96	11.8	37.5	(3) 7.79	11.3	36.3			
	M	8.20	12.6	39.0	7.12	10.4	33.3			
	FA	5.49	8.6	33.0	6.60	8.4	33.0			
	FV	4.35	7.7	33.0	7.17	8.8	34.1			
9	P	(1) 7.69	12.0	38				(2) NORMAL CONTROLS 8.30	12.1	39.2
	M	7.60	11.6	39						
	FA	9.76	10.4	--						
	FV	9.32	9.8	--						

P- Preliminary; M - Maternal Terminal; FA - Fetal Umbilical Artery; FV - Fetal Umbilical Vein
a - Erythrocytes in millions/cmm; b - Hemoglobin in grams/100 ml; c - Packed cell volume/100ml
Numbers in parentheses indicate number of animals used to obtain averages.

Table 3. Leukocyte Determinations.

Stage of Gestation in months	Sample Source	Non-Infected						Intraperitoneal Infection						Intra-Uterine Infection					
		WBC[a]	Differential %[b] E	S	P	L	M	WBC[a]	Differential %[b] E	S	P	L	M	WBC[a]	Differential %[b] E	S	P	L	M
		(1)						(1)											
6	P							8150	0	1	29	59	5						
	M	6600	23	8	27	35	7	9750	3	3	29	63	2						
	FA	4400	2	3	12	83		9100	3	4	32	61							
	FV	4200	2	1	13	79	5	7100	3	5	26	66							
								(3)						(1)					
7	P							10190	7	4	24	62	3						
	M							10080	10	2	18	67	3	5150	3		23	65	9
	FA							6380	2	2	17	77	2	6990*		6	3	87	4
	FV							5550	2		16	81	1	6777**		1	2	95	2
		(1)						(3)											
8	P	11380	18	1	12	66	3	11220	18	3	30	47	2						
	M	10950	9	1	24	60	6	8940	15	1	26	54	4						
	FA	6250	3		16	80	1	6500	5	2	31	59	3						
	FV	6100	5	5	23	65	2	6980	6	3	30	57	4						
		(1)												(2) NORMAL CONTROL					
9	P	9200	5	5	26	61	3							9200	3	4	23	62	3
	M	8250	5	7	40	44	4												
	FA	10150	2	4	38	55	1												
	FV	9550	1	4	35	58	2												

E - Eosinophil; S - Stabs (immature PMN); P - Polymorphonuclear leukocytes; L - Lymphocytes; M - Monocytes.
a - White blood cells/cmm; b - % of various leukocytes/100 WBC.
* - Corrected for nucleated RBC 126/100 WBC; ** - Corrected for nucleated RBC 140/100 WBC.

Table 4. Blood Gas Determinations.

Stage of Gestation in months	Sample Source	Non-Infected Volumes % O_2	Non-Infected Volumes % CO_2	Intraperitoneal Infection Volumes % O_2	Intraperitoneal Infection Volumes % CO_2	Intra-Uterine Infection Volumes % O_2	Intra-Uterine Infection Volumes % CO_2
6	P			(1) 10.4	48.5		
	M			10.5	50.5		
	FA			0.0	53.8		
	FV			4.3	49.5		
7	P			(3) 10.3	(3) 51.0	(1)	(1) 44.4
	M			12.1	42.3	14.8	49.7
	FA			0.25	49.4	3.5	43.5
	FV			3.7	46.9	1.0	44.1
8	P	(1) 15.6	(1) 50.9	(3) 7.5	(3) 51.9		
	M	16.0	46.5	13.9	49.6		
	FA	0.0	31.1	3.3	50.2		
	FV	0.7	47.0	4.1	50.1		
9	P	(1) 12.2	44.4			(2) NORMAL CONTROL 14.1	45.5
	M	13.5	49.7				
	FA	1.8	43.5				
	FV	5.0	44.1				

Table 5. Nitrogen Determinations.

Stage of Gestation in months	Sample Source	Non-Infected NPN* mg %	Non-Infected Creatinine mg %	Intraperitoneal Infection NPN* mg % v	Intraperitoneal Infection Creatinine mg %	Intra-Uterine Infection NPN* mg %	Intra-Uterine Infection Creatinine mg %
		(1)	(1)	(1)			
6	P			20.8	1.90		
	M	39	2.25	--	2.25		
	FA	48	4.30	--	4.00		
	FV	54	4.20	--	3.20		
				(2)	(3)	(1)	(1)
7	P			20.9	1.89		
	M			21.7	2.17	34.5	1.8
	FA			24.9	3.90	57	2.7
	FV			29.1	4.00	43	2.3
		(1)	(1)	(3)	(3)		
8	P	32	2.12	25.2	2.12		
	M	27	2.30	23.4	2.50		
	FA	29	4.50	28.0	4.31		
	FV	24	4.30	30.9	4.75		
		(1)	(1)			(2) NORMAL CONTROL	
9	P	23.3	2.10			22.7	1.95
	M	21.6	2.25				
	FA	30.0	3.15				
	FV	36.0	3.45				

* NPN is Non-Protein Nitrogen.

Table 6. Glucose Determinations.

Stage of Gestation in months	Sample Source	Non-Infected	Intra-Peritoneal Infection	Intra-Uterine Infection
		Glucose mg %	Glucose mg %	Glucose mg %
6	P	(1)	(1) 69	
	M	60	130	
	FA	350	212	
	FV	316	212	
7	P		(3) 80.5	(1)
	M		75.3	100
	FA		212	260
	FV		216	280
8	P	(1) 72	(3) 84	
	M	72	107	
	FA	139	204	
	FV	134	193	
9	P	(1) 74		(2) NORMAL CONTROL
	M	71		71
	FA	147		
	FV	170		

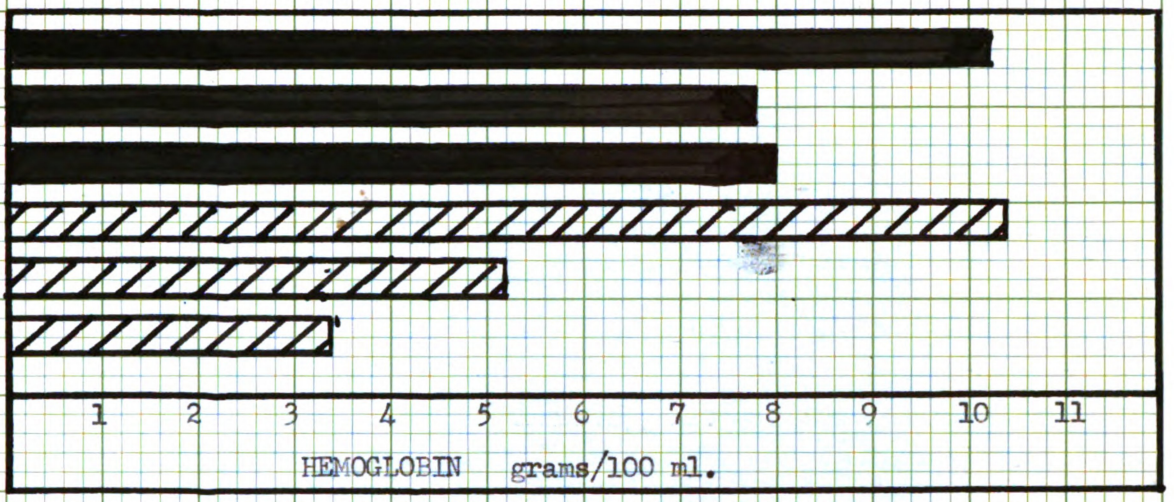
Table 7. Fetal Respiratory Quotients.

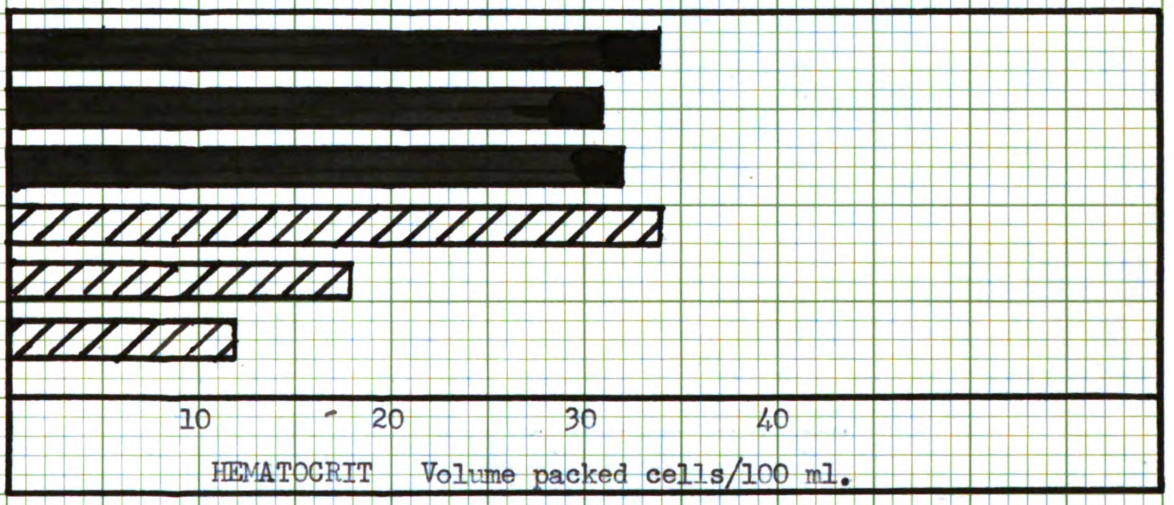
Age of Fetus	Sample Source	Volumes % O_2	Volumes % CO_2	R.Q.*
6 months infected	Umbilical Artery	0.0	53.8	
	Umbilical Vein	4.3	49.5	1.00
7 months infected	Umbilical Artery	0.25	49.4	
	Umbilical Vein	3.7	46.9	0.704
9 months non-infected	Umbilical Artery	1.8	48.5	
	Umbilical Vein	5.0	44.1	1.05

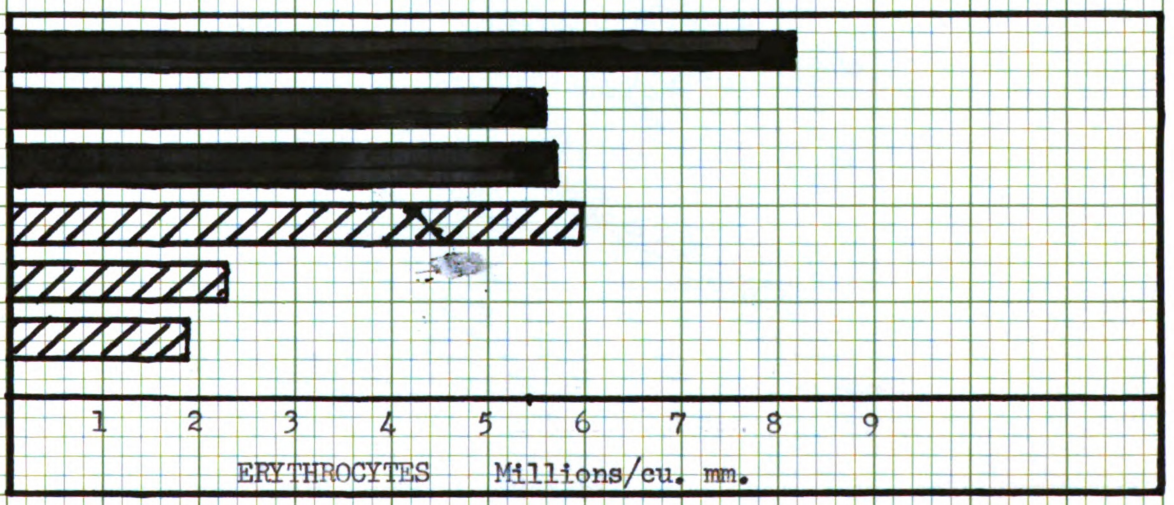
$$* \text{ R.Q.} = \frac{\text{Venous-Arterial } CO_2 \text{ Volumes \%}}{\text{Arterial-Venous } O_2 \text{ Volumes \%}}$$

CHART I

COMPARISON OF FETAL ERYTHROCYTE CHANGES PRODUCED BY 2 METHODS OF INFECTION







Intraperitoneal Infection

Intra-uterine Infection

MA Maternal Artery
FA Fetal Artery
FV Fetal Vein

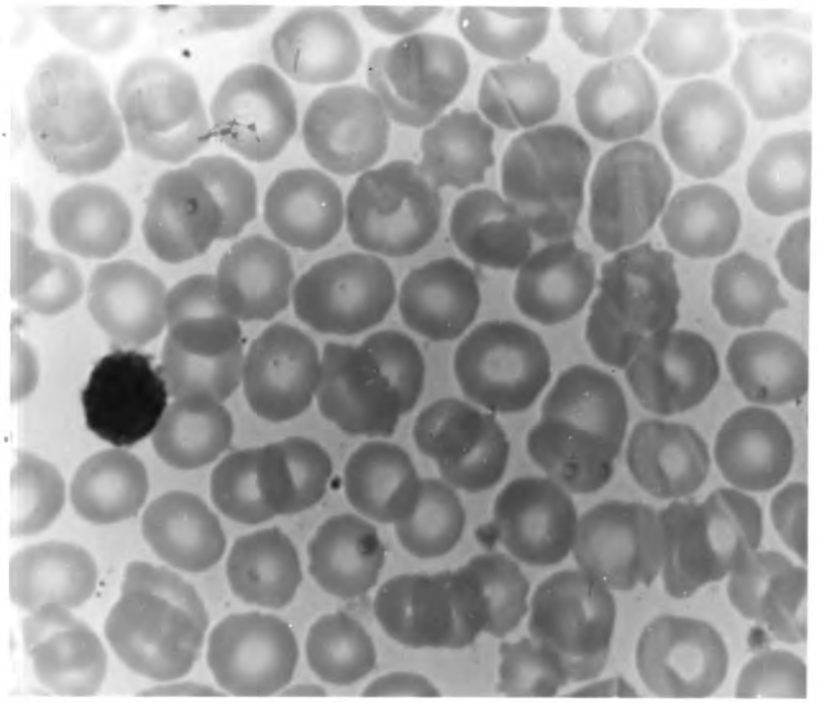


Figure 1. Blood smear from the sixth month fetus of a dam infected intraperitoneally with Leptospira pomona. Cells appear normal in size and shape.

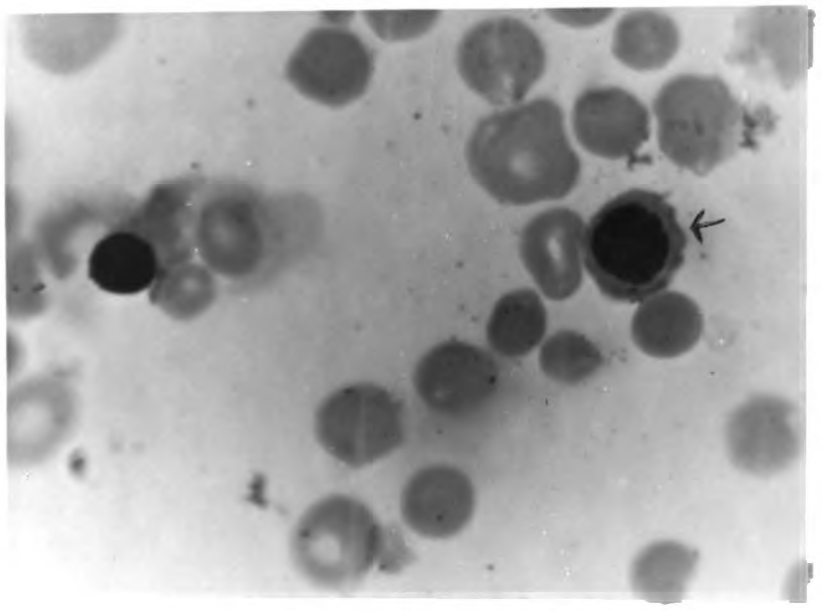


Figure 2. Blood smear from the sixth month fetus of a dam infected intrauterinely with Leptospira pomona. Note anemia, poikilocytosis and anisocytosis. Arrow indicates normoblast (nucleated red cell).

DISCUSSION

In general, the values obtained would seem to substantiate observations made by workers in several different fields. No significant change in levels between non-infected and intraperitoneally infected pregnant heifers was found. These values, in turn, are not outside the range established for normal, non-pregnant cattle. This agrees with published reports on blood values in pregnant cattle used in experimental work on other bacterial diseases. It also would appear to support the workers who found few clinical symptoms in cattle infected with L. pomona in the last trimester of pregnancy.

The variations between samples obtained from the umbilical artery and the umbilical vein are either too slight or too inconsistent to be of any significance except in the case of the blood gases. The main purpose in collecting both arterial and venous samples was, in fact, to compare these blood gases so as to assess the effect of a possible leptospiral hemolytic toxin. It must be kept in mind that the fetal circulation is the reverse of the normal adult circulation, with oxygenated blood carried in the umbilical vein and the arterial blood actually containing reduced hemoglobin and carbon dioxide which is being returned to the maternal circulation.

The values given in standard clinical texts for most animal species have very wide ranges which may be due to a

great variation among different breeds. Most of the values determined in this work fall in a much narrower range as would be expected with animals of the same breed. The blood gas determinations were the most variable, and since these are the samples most affected by external factors, this is not too surprising. Reineke, et al. (36), found that excitation, which increases the carbon dioxide:oxygen ratio could be prevented by anaesthetizing the animal before obtaining the blood samples. On the other hand, excess anaesthesia must also be avoided since this leads to apparently reduced respiratory quotients. Dawes and Mott (9) likewise list anaesthesia among the causes of the great variation they observed in their oxygen values. Shaw (38) found large fluctuations in both blood gases when the samples were taken within 15-20 minutes after anaesthetization. Since our fetal values had to be obtained on viable animals, it was necessary to obtain both maternal and fetal samples long before this desirable time lag could occur. Some difficulties were also encountered with certain heifers who were refractory to tranquilizers and anaesthesia. Naturally, too, a few blood samples met with such seemingly unavoidable accidents as clotting or even leakage or droppage during the trip from barn to laboratory. This accounts for the blank spaces found in the tables or for certain averages being computed on a smaller number of tests.

The respiratory quotients given in Table 7 are the only three sets of fetal values which fall in a range permitting this analysis. The fact that even these few comparisons

could be made is encouraging and proves the techniques are workable if more and better samples could be obtained. The small number of animals available limits the possible interpretations of many of the determinations. These quotients are within the limits found by other workers on maternal blood samples. Unity is an approximate median for these quotients with some workers reporting from 0.5 to 2.0. The great disparity in maternal and fetal oxygen values indicates that the much lower fetal oxygen value contributes to what one author calls the oxygen avidity of the fetus, and the very low values in certain cases (6 and 7 month infected and 8 month non-infected) argues for almost complete utilization of all available oxygen by the fetus.

The fetal glucose and nitrogen values are higher than the maternal values in all but one case (non-protein nitrogen in the 8th month non-infected heifers). There is no published data to account for the nitrogen results, but bovine glucose concentrations may follow the pattern discussed by Huggett, et al. (26), in their sheep studies. In the commonly used methods of glucose determination, including the Folin and Wu procedure used in this paper, a combination of reducing substances is actually being measured. This includes a variable amount of fructose. They feel that the maternal and fetal glucose concentrations should be approximately equal, with the apparent excess in the fetal blood representing the amount of fructose present. Glucose is diffusable through the placental membranes in either direction, but fructose can only go from mother to fetus.

It can, however, be readily produced by the fetus from glucose and may be utilized better in this form by the fetus. Goodwin (23), who has divided most mammals (including whales) into two groups according to the concentration of fructose in the blood of the fetus, finds appreciable fructose in the calf at birth and shortly after. This work illustrates again the use of the word fetus when the experimental animal is actually new-born.

The hematological values substantiate certain predictions and observations. The leukocyte counts showed no appreciable change in either total numbers per unit volume or in the percentages of the different types of white cells for any of the specimens: fetal or maternal, infected or non-infected. This would indicate that the infection was not of the severe, acute type which causes greatly increased numbers of cells with an absolute increase of immature polymorphonuclear leukocytes.

The maternal erythrocyte, hemoglobin, and hematocrit values likewise showed little deviation in infected and non-infected heifers. The fetal values seemed unaffected by the intraperitoneal maternal infection, but in the fetus of the intra-uterinely infected heifer, the dramatic changes shown in Chart 1 occurred. This demonstrates that when the leptospiral organism actually enters the uterus, the fetus is definitely and severely affected, with a drop in hemoglobin concentration of almost 50% from an average venous and arterial concentration of 8.5 grams/100 ml of blood to 4.3 grams/100 ml. The hematocrit, in like manner, dropped from

a packed cell volume of 33 ml/100 ml of blood to only 16 ml/100 ml, again averaging the fetal venous and arterial values. The total erythrocyte value, dropping from an average of 6,630,000/cu.mm. to 2,080,000/cu.mm. appears to be the most severe change of the three determinations, but this estimation is also the one with the greatest inherent error. Therefore, the hemoglobin and hematocrit values are a truer representation of events.

In Figure 1, a blood smear from the 7 month fetus of intraperitoneally infected heifer #10, the erythrocytes appear normal in size and shape. Figure 2, a similar smear from the 7 month fetus of intra-uterinely infected heifer #13, exhibits marked poikilocytosis and anisocytosis. The arrow indicates a normoblast (nucleated erythrocyte), an immature cell released by the bone marrow into the peripheral circulation because of hypoxia caused by the great destruction of circulating erythrocytes. As indicated in Table 3, the two fetal samples from heifer #13 averaged 133 normoblasts per 100 leukocytes.

To draw the obverse correlation, since the fetal blood concentrations of these various constituents were not affected by the intraperitoneal infection of the dam, it would appear that no toxin crossed the maternal barrier.

Naturally, an abortion, or abortions, would have provided answers more on the positive side, but the small numbers of animals, and the low expectancy rate of abortions in leptospiral infections did not favor this occurrence.

Bauer's (6) work with an hemolytic exotoxin in lambs,

and further studies with this hemolysin in our laboratory in pregnant ewes, strongly suggests that this substance is the factor responsible for the erythrocytic destruction leading to jaundice, hematuria and renal damage of the classic, acute forms of leptospirosis. Strains of L. pomona have been shown to vary in their hemolytic activity and this, along with individual physiological differences as previously discussed, may account for the wide variations in symptoms in leptospiral infections due to the pomona serotype.

Our next step, when animals are available, will be to try this hemolysin on pregnant heifers to see if they follow the same pattern as the ewes, or if this marked red cell destruction has some species specificity due to increased erythrocyte fragility.

SUMMARY

The intraperitoneal infection of seven pregnant heifers with L. pomona failed to produce any significant change in fetal blood values in a selected group of ten chemical and hematological determinations. The intra-uterine infection of one pregnant heifer with the same strain resulted in a definite reduction in the fetal erythrocyte count, hemoglobin content and volume of packed erythrocytes. The remaining blood values were not affected in this fetus. These results would appear to negate the hypothesis that leptospiral bovine abortion is produced by death of the fetus due to a toxin which is capable of crossing the placental membrane.

This small series of determinations establishes a range of bovine fetal blood values during the sixth to ninth month of uterine life.

BIBLIOGRAPHY

1. Albritton, Erret C. (1953) Standard Values in Blood. W. B. Saunders Co. Philadelphia.

2. Alston, J. M. and J. C. Broom (1958) Leptospirosis in Man and Animals. E & S Livingstone Ltd, Edinburgh.

3. Barclay, A. E., K. J. Franklin and M. M. L. Pritchard (1945) The Foetal Circulation. Charles C. Thomas, Springfield.

4. Barcroft, J. and D. H. Barron. (1942) Circulation in the Placenta of the Sheep. J. Physiol. Proceedings. 100 20P.

5. Barron, D. H. (1944) The Changes in the Fetal Circulation at Birth. Physiol. Rev. 24:277-295.

6. Bauer, D. C. (1959) Virulence Factors of Leptospira pomona. Ph. D. Thesis. Michigan State University.

7. Cartwright, George E. (1958) Diagnostic Laboratory Hematology. 2nd ed. Grune and Stratton, New York.

8. Coffin, David L. (1945) Manual of Veterinary Clinical Pathology. Comstock Publishing Co., Inc. Ithaca, N.Y.

9. Dawes, G. S. and J. C. Mott. (1959) Increase in Oxygen Consumption of the Lamb after Birth. J. Physiol. 146:295-315.

10. Dawes, G. S., J. C. Mott and J. G. Widdicombe. (1954) The Foetal Circulation in the Lamb. J. Physiol. 126: 563-587.

11. Dukes, H. H. (1947) The Physiology of Domestic Animals. 6th ed. Comstock Publishing Co., Inc. Ithaca, N. Y.

12. Edwards, M. J. and B. B. Ross. (1959) Graphical Representation of Respiratory Gas Dissociation Characteristics of Fetal and Maternal Blood. J. Applied Physiol. 14:454-456.

13. Fennestad, K. L. and C. Borg-Peterson. (1956) Studies on Bovine Leptospirosis and Abortion. I. Serological Examination of Aborting and "Normal" Cattle. Denmark Nord. Vet. Med. 8:465-480.

14. Ibid. (1956) II. Experimental Leptospirosis in Pregnant Heifers. Nord. Vet. Med. 8:815-833.

15. Ibid. (1956) III. Attempts to Demonstrate Leptospira in Cotyledons from Aborting Cattle. Nord. Vet. Med. 8:822-836.

16. Ibid. (1958) IV. Demonstration of Leptospira in Foetuses from Field Cases of Abortion in Cattle. Nord. Vet. Med. 10:302-308.

17. Ibid. (1958) Fetal Leptospirosis and Abortion in Cattle. J. Inf. Dis. 102:227-236.

18. Ibid. (1958) Pathogenesis of Leptospiral Bovine Abortion. Symposium on Leptospira. Lublin, Poland.

19. Ferguson, L. C., M. R. Irwin and B. A. Beach. (1941) The Effect of Parturition on the Blood Picture of Cows in Health and during Infection with Brucella abortus. Am. J. Vet. Res. 2:394-399.

20. Ferguson, L. C., J. C. Ramge and V. L. Sanger. (1957) Experimental Bovine Leptospirosis. Am. J. Vet. Res. 18:45-49.

21. Galton, Mildred M. (1959) The Epidemiology of Leptospirosis in the United States with Special Reference to Wild Animal Reservoirs. The Southwestern Veterinarian. 13:37-42.

22. Gochenour, W. S. and R. H. Yager. (1953) Manifestations of Bovine Leptospirosis. Vet. Med. 48:218-220.

23. Goodwin, R. F. W. (1956) Division of the Common Mammals into Two Groups According to the Concentration of Fructose in the Blood of the Foetus. J. Physiol. 132: 146-152.

24. Hawk, P. B., B. L. Oser and W. H. Summerson. (1947) Practical Physiological Chemistry. 12th ed. Blakeston Co. Philadelphia.

25. Huggett, A. St. G. (1926) Factors Influencing the Foetal Respiratory Centre. J. Physiol. 61:30-31.

26. Huggett, A. St. G., F. L. Warren and M. V. Warren. (1937) The Origin of the Blood Fructose of the Foetal Sheep. J. Physiol. 113:258-275.

27. Kaiser, I. H. (1959) The Measurement of Fetal Oxygen. Am. J. Obs. and Gyn. 77:225-263.

28. Keys, Ancel B. (1934) The CO_2 Balance between the Maternal and Foetal in the Goat. J. Physiol. 80:491-501.

29. McCarthy, Earl F. (1934) A Comparison of Foetal and Maternal Haemoglobin in the Goat. J. Physiol. 80:206-212.

30. Metcalfe, J., S. L. Romney, J. R. Swartwout, D. M. Pitcairn, A. N. Lethin, and D. H. Barron. (1959) Uterine Blood Flow and Oxygen Consumption in Pregnant Sheep and Goats. Am. J. Physiol. 197:929-934.

31. Morse, E. V. and S. H. McNutt. (1956) Experimental Leptospirosis. I. The Course of Leptospira pomona Infection in Pregnant Heifers. J. A.V.M.A. 128:225-229.

32. Morse, E. V., R. L. Morter, R. F. Langham, A. Lundberg a and D. E. Ullrey. (1957) Experimental Ovine Leptospirosis, Leptospira pomona Infection. J. Inf. Dis. 101:129-136.

33. Morter, R. L. (1960) Studies on Bovine Leptospiral Abortion. Ph. D. Thesis. Michigan State University.

34. Morter, R. L., R. F. Langham, and E. V. Morse (1958) Experimental Leptospirosis. VI. Histopathology of the Bovine Placenta in Leptospira pomona Infections. Am. J. Vet. Res. 19:785-791.

35. Peters, J. P. and D. D. VanSlyke. (1932) Quantitative Clinical Chemistry. Vol. II Methods. Williams and Wilkins, Baltimore. 324-328.

36. Reineke, E. P., C. D. Stonecipher and C. W. Turner. (1941) The Relation between the Fat and Carbohydrate Metabolism of Lactation as Indicated by the Respiratory Quotient of the Mammary Gland. Am. J. Physiol. 132: 535-541.

37. Roos, J. and C. Romijn. (1938) Foetal Respiration in the Cow. J. Physiol. 22:249-267.

38. Shaw, J. C. (1946) Lactic Acid, Pyruvic Acid, Amino Acids, Acetone Bodies, O_2, CO_2 and Hemoglobin in Arterial and Mammary Venous Bloods of Cows under Various Physiological Conditions. J. Dairy Science. 29:183-197.

39. Stoenner, H. G. (1954) The Management of Herds Affected by Bovine Leptospirosis. Proc. Am. Vet. Med. Ass. 83-87.

40. TePunga, W. H. and W. A. Bishop. (1953) Bovine Abortion Caused by Infection with Leptospira pomona. New Zealand Vet. J. 1:143-149.

41. Turnbull, E.P.N., and J. Walker. (1955) Haemoglobin and Red Cells in the Human Foetus. II. The Red Cells. Archives Disease in Childhood. 30:102-110.

42. von Desec, D. (1929) Beitrag zur Kenntnis der fetalen Blutentwicklung beim Rinde. Pfluger's Archives. 221: 321-333.

43. Walker, J. and E.P.N. Turnbull. (1955) Haemoglobin and Red Cells in the Human Foetus. III. Foetal and Adult Haemoglobin. Arch. Dis. Childhood. 30:111-116.