Final Report on the Animal Blood Typing Laboratory, UNESP "Julio de Mesquita Filho", Faculdade de Ciéncias Agrárias e Veterinárias "Campus" de Jaboticabal, São Paulo, Brasil; August 1978 to August 1979

by

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The development of the laboratory was very slow by North American standards. Necessary equipment and supplies were never completed during the year. An inordinant amount of time was spent in hunting for equipment and supplies, and in devising substitutes and the means to acquire them.

A reasonably good rabbit facility had been constructed and was ready on my arrival, although the caretaker problem was never satisfactorily solved. The cattle bleeding facilities and number and variety of cattle available to us for absorption and immunizations were inadequate. The lack of a technician until late in the program and the lack of a secretary diminished research results considerably. Probably the most serious fault was the lack of time available to the main trainee, Ana Isabel de Assis. She was teaching, or consulting with students all but the last 6 weeks.

In spite of these difficulties, a functioning immunogenetics laboratory was developed. Approximately 250 cattle were tested with ISU reagents, plus a few reagents from Texas A & M University and the University of Wisconsin. These blood types were submitted to Diretor Giannonni periodically. A preliminary breed summary also was made of red cell factors and phenogroup frequencies for Nelore, Gir, Guzerá, Red Sindhi, Santa Gabriela, and Caracu breeds. This data also was reported periodically to Director Giannonni and to Professor D. S. Robertson, chairman of the Department of Genetics, ISU, Ames, Iowa.

The antisera and reagent bank is the most direct indication of the progress made in setting up the laboratory. Thirty rabbits were immunized with blood from seven cattle (5 Nelore and 2 "Hollandes"). Eight of these antisera remain to be analyzed. The others were absorbed to make seven or eight reagents: A, D, Z', Z'+X_1, "GY_2E_1'"?, F, V, Z+Z'. The lack of sufficient variety for absorbing cells is the main reason some reagents were not polished.

Forty-three cattle were immunized. Fifteen did not respond with antibodies reactive with rabbit complement. Twenty eight did so respond, or had naturally strong antibodies, and were bled in quantity. These isoimmunizations after analysis and absorptions so far have resulted in 17 reagents of which 9 were more or less standard and six were probable new factors. Further work is necessary to prove or polish these reagents: A, G^+, Y^-, Y_1?, Y^-, B'_1?, G'_1, C_1^+, R_1, X_1, NF1, 2+, 3+, 4, NF + R_1, NF + (CS1).

Summary conclusions and comments on the blood types of the Brazilian cattle:

1. G'_2 new factor from and ISU reagent was proven by sire family data to fit into the B system, and 14 different B phenogroups were demonstrated to possess it (see sire family of Imrath, Binag, Lord Krisna, Gaiado, and Majarico).

2. Almost all factors known in cattle were encountered. Since no laboratory has all the known reagents, the reservations or qualifications to the above statement reside mainly in the difficulty in distinguishing Y_1 & Y_2, J' & K', M_1 & M_2, U_1 & U_2, the F and V subtypes, and finally, there was no reagent for factor .

3. Subtypes of factor D were proven, but the number and internal relationships have not been clarified. Six D subtypes are possible from the data so far.

4. F & V subtypes were relatively common in Nelore, Guzerá, Gir, rare in Caracu, and not encountered in the 19 Red Sindhi.

5. Factor Z' without A in the A system phenogroups was demonstrated in Nelore (see Hanuman, Hinduppur, and Imrath sire families) and Gir. Such a phenogroup was suspected by Stormont many years ago. H without D in A system phenogroups also was demonstrated (e.g. N2266).

6. Caracu have the highest frequency of U in any known breed.

7. Caracu have at least 10/23 = 43% B phenogroups in common with Texas Longhorns (and possibly as many as 13/23 = 57%) demonstrating their Iberian relationship.

8. All B types in Guzerá were capable of being phenogrouped by known European-American cattle B groups! The superficially obvious conclusion that they are closely related is patently false. But no other 7 or 8 Zebu samples could have been selected to fit such phenogrouping. Guzerá B types need particular attention in this respect in more extensive research.

9. There were 61 presumptive B phenogroups <u>new</u> in cattle blood typing as compared to the University of Texas print-out sheet. The numbers are indicated by breed and frequency in the table below:

Number of Animals	Breed	Number of B Phenogroups Total	NEW	Percent NEW
40	Caracu	23	10	43%
50	Gir	16	15	94%
7	Guzerá	12	0	0 !
68	Nelore	36	29	81%
19	Red Sindhi	11	7	64%
10	Santa Gabriela	14	6	43%
194		112	67	

Only two B phenogroups in Gir were found in other Indian Breeds of this sample; only one in Nelore, and only one in Red Sindhi. Santa Gabriela were synthesized from other existing breeds.

10. I made Network Pedigrees of cattle at the Aripuanã Research Station in far northern Mato Grosso, and of the cattle listed in number 8 above. This method enabled the research personnel and administrators to vizualize more easily the actual breeding structure of the herds.

In addition to the above more orthodox manipulations, research efforts were made in two new directions. (This research area especially was diminished by the teachers strike for more pay in the state of São Paulo, the effects lasting well beyond the end of the strike.)

1. Three new species were used as recipients for cattle blood.
   A. There were 24 hogs of selected breeds and crosses immunized and the resulting antisera frozen.
   B. Several dogs were immunized and the antisera stored frozen.
   C. Several horses were immunized and the antisera stored frozen.

All these species responded with high titered species-specific antisera. These efforts were not very far along by the end of the designated year. But a few absorptions demonstrated that antibodies for individual differences also were contained in the immune sera.

2. Complement from rabbits, guinea pigs and hogs was contrasted with various reagents. As suspected by Stormont and demonstrated by Miller (unpublished), Ana Isabel de Assis confirmed that the complement sources differed in their reaction with the cattle red cells sensitized, and she extended such observations to hog complement. While some reagents plus reactive cells reacted with complement from any of the three sources, others differed in strength, or even in absolute reaction or lack of it with the different complements. The textbook implication that complement is non-specific in that it <u>will react</u> with <u>any sensitized system</u> is clearly false and requires clarification.

General Conclusion:

In spite of the demonstrated successes, research potential, and known utility of the blood typing test in parentage analysis, twin diagnosis and individual identification, the prospect for continuation of the laboratory at Jaboticabal is rather precarious because of the minimal financial support and incomplete training. It has been demonstrated that such minimal funds will still allow the laboratory to function slowly, if there is willing and forthright cooperation by other departments and personnel. Without one or the other or both, a merging with the laboratory at São Carlos might be contemplated.

Laboratory Personnel:

Leader: Dr. Wilmer J. Miller, ISU, Ames, Iowa

Trainee: Ana Isabel de Assis

Students: Miriam Luz Giannonni
Claudimir de Carvalho
Domingos Cruz Filho
Luzia Helena Queiroz

Funcionarios: Shirley Panosso Paimundo
Dirce do Nacimiento
José Edson Fernandes

Recommendations:

At a minimum the laboratory should have two persons trained to conduct the tests and immunizations. Ordinarily in the USA this is a blood grouping specialist and a trained technician, or two blood grouping specialists. The laboratory is severely hampered if a new technician or colleague has to be trained at short intervals. It really takes about two years of full time work (US style) to train into self directed, independent blood typing. If possible, a return of myself, or another specialist should be contemplated.

The Animal Blood Typing Laboratory at Jaboticabal should continue absorptions and analysis of anitsera not yet finished. Additional immunizations should be continued in cattle and the other species. After about 50 standard reagents are replicated in sufficient quantity to last for 10-20 years, service typing or commercial blood typing should be started to make the laboratory independent financially, while further reagent preparations and research continue until the laboratory can enter the world wide comparison tests.

Since parentage analysis is an important part of service typing, the blood group specialist needs good genetic background in order to be accurate and speak with authority on the more difficult analyses, as well as comprehending the general picture. (Indeed, few professionals can adequately distinguish the rather simple classes of epistasis and dominance which are now becoming more widely known in blood grouping.) Therefore, I recommend that the trainee, Ana Isabel de Assis, spend at least 4-6 months in the USA at an appropriate institution. A year would be even better, enabling her to do some research. She could sit in on my course for basic genetics and in an advanced course in biochemical genetics.

Further, it would be advantageous for her to visit the major cattle blood typing laboratories in the USA to note the different directions of research and methods used. The current service typing laboratory at College Station, Texas (Dr. Don Weseli), the "parent" lab. at Madison, Wisconsin (now under Dr. W.H. Stone), the lab. now emphasizing biochemical approaches at Columbus, Ohio (Dr. H.C. Hines) and Dr. Stormont's lab. at Davis, California should be considered.

Ana Isabel de Assis has demonstrated outstanding capability, intelligence and willingness to work hard. She certainly should be extended the sustained assistance and support that this position requires.

Other academic events appropriate to mention:

1. Two weeks were spent in La Plata, Argentina, 5-18 Nov 78 in attendance of the Sextas Jornadas Internacionales de la Facultad de Ciências Veterinarias. I presented an invited paper on some wild type and genetic considerations of the North American Longhorn, and was coauthor of another paper "Segregation of blood phenogroups in the Argentine "Bovinos Criollos".

2. I presented a 90 minute invited lead paper on blood groups in domestic animals at the III Semana de Zootecnia at Pirassanunga, Sao Paulo, 11 Dec 78.

3. The Sociedade Brasileira de Bioquímica VIII Reunião Anual held a third convention on 18-21 April 79 at Caxambú, Minas Gerias, Brasil. I presented a "posterboard" on "Evidence from lectin reactions for mandatory pairing of the terminal sugars in human blood group AB".

4. I gave about 12 90-minute informal lectures on classical genetics and Immunogenetics to staff and students on the Jaboticabal campus.