

Session 9

Workshop Summary

Chairperson: Dr. Keith Betteridge, University of Guelph, Ontario, Canada

Summary of Workshop Proceedings

K. Betteridge

OUR BRIEF FOR THIS CLOSING SESSION HAS BEEN VERY clearly set for us by Drs. Powell and Tobin. With a view to defining “where we go from here,” we have been asked to center our discussion on three general areas:

- the approaches that seem most promising for identifying potential causative agents of Mare Reproductive Loss Syndrome (MRLS);
- suggestions for a useful laboratory model of the syndrome;
- cost-effective preventative measures for MRLS and for communicating these to all those affected by the condition.

I say “our” brief because you are going to do the work; I’m just the chair!

Because my job is not to summarize the meeting, I’m going to ask the summarizers of the various sessions to come forward and be available to answer questions as they crop up. However, before throwing the discussion open to the summarizers and to you on the floor, I must say that it would be churlish indeed not to take this opportunity to make some very appreciative comments about the meeting in general before getting down to the specifics of our mandate.

All of us have been very conscious of the fact that, as was said yesterday at Keeneland, there is nothing good about MRLS. Those of us coming from outside feel very sympathetic and very sad about those dreadful pictures we’ve been seeing of the problems you have been suffering over the past two years. I repeat, there is nothing good about MRLS; and yet, paradoxically, from the scientific point of view, this has been a most interesting workshop. As I’ve heard so many people say as we have been eating and talking outside, “If it weren’t for its devastating effects, this disease would be great fun to investigate.” But fun, it is not; important, it most certainly is.

Dean Smith set the scene for us in saying how people in Kentucky have been exemplary in three aspects of investigating MRLS: the investigation has been multi-disciplinary, it has been collaborative, and there has been great communication, both among the investigators and to the affected public.

Dr. Webb said how skeptical he was about this workshop—“How could it serve any useful purpose?”—and yet he has changed his mind during the meeting, and I heard him say that he sees it as a “positive move to integration.” His words, I think, are evidence of the success of the

initiative of Drs. Powell and Tobin and their colleagues in calling us all together.

As an outside observer, I’ve been enormously impressed by the efforts that have already gone into identifying potential causative agents. There has been what seems to me a most remarkable collaboration between the practitioners in the field and investigators in the laboratory. Needless to say, none of the investigations that we have heard about could have been done, nor could the whole picture have been characterized, but for the remarkable input of all the practitioners in the affected areas of Kentucky, and I think they deserve special praise for that.

There has also been commendable collaboration between various departments of the University of Kentucky and between the University and government in the investigation of MRLS, as was brought out very clearly at today’s lunch.

Are eastern tent caterpillars (ETC) involved in MRLS? I would say that the consensus that is emerging from this meeting is that they are involved, although this is not universally accepted, and I think it is something that still needs to be debated. There have been interventions from the floor about infestations apparently in the year 2000, though others contend that they were nothing like as extensive or intense as in 2001. So, I don’t think that the role for ETC is absolutely cut and dried. Remember, too, that Dr. Kronfeld’s statistical summaries leave some doubt as to the strength of some of the experimental evidence for caterpillar involvement. In this connection, of course, we need to recognize the enormous difficulty of getting statistical significance out of the kind of numbers that we can work with—a key problem that we have identified and must cope with in the future.

If ETC are involved, then we have to know a lot more about them. Dr. Sebastian referred to ETC as “God’s little creatures,” quoting a Web site description of them. I went to William Shakespeare, *Richard II*, Act II:

“... Bushy, Bagot and their complices,
The caterpillars of the commonwealth,
Which I have sworn to weed and pluck away.”

(I thought that this was especially appropriate when I came past the Commonwealth Stadium on the way here).

Ontario Veterinarian College, University of Guelph, Guelph, Ontario, Canada.

So, Bushy, Bagot, and all their friends certainly need to be better understood. If they are involved, the question is “How?” That question has been the focus of discussions—and I think very, very useful discussions—throughout the workshop. We heard about placentitis and funisitis; are they caused by a bacteremia from commensals, and, if so, is that bacteremia being initiated through the cervix or in some other way—through irritation of mucous membranes, perhaps? In fact, the portal of entry seems to be a key question that needs to be answered. Dr. Benirschke told us that in humans the portal of entry for any placentitis is via the cervix. In that context, I was intrigued to hear that the amniotic fluid seems to become infected and that infection doesn’t seem to spread away from the amnion. Perhaps we need to remember that in all the talk of the placenta that we have heard over the past two days, we have really been talking about the fetal membranes. There are two sides to a placenta—the maternal and the fetal sides. I don’t want to be pedantic, but I think that the fact that we don’t really know anything (or very little) about what’s happening on the maternal side of the placenta needs to be borne in mind, a point to which I will return in considering potential models of MRLS.

Again, in relation to fetal fluids, I think it’s perhaps salutary to remember that the accumulation of fetal fluids is a very dynamic process, at least in pigs (1). Do analogous peaks in the accumulation of these fluids occur in mares? If so, might they indicate that there are portals opening between the conceptus and the mother, and might any opening of such portals constitute a route of entry for bacteria? The possibility that fluid movement might be important in MRLS is supported by the fact that the associated pericarditis also involves an accumulation of fluid over serous epithelia. Perhaps getting back to the basic physiology of how and when equine fetal fluids accumulate, expanding the studies of Arthur (2), could be useful, at least from an epidemiological point of view.

Much as been made of the fact that there have been no losses experienced in mares pregnant less than 35 days. I find this very interesting indeed, because I’m most interested in the conceptus up to 35 days. I would like to point out that it would be wrong to go away with the impression that there is no placental exchange before 35 days; there is, of course, but the nature of that exchange is very different, involving the bilaminar omphalopleure probably up until, and certainly after, the capsule disappears at about day 21. The rate of expansion of the conceptus should remind us all that there is exchange between the mother and conceptus right from the beginning of pregnancy, so don’t let’s forget that in thinking about the etiology of MRLS.

Certainly, there has been much thought about early detachment of the placenta being involved in MRLS. Dr.

LeBlanc, for one, is a very strong advocate of this as being one of the ways in which the whole syndrome comes about. In relation to that, I wonder whether there is a place for some more detailed ultrasonography. I’m thinking here of Doppler ultrasonography that is being used to such good effect by Dr. Bollwein in Germany to measure blood flow to the uterus. It strikes me that if we do have a reproducible model—and it would have to be a reproducible model in the horse and not a laboratory animal—that perhaps getting at the changes in blood flow to the uterus could be very important. I think that the only sign of fetal distress that I heard about during the presentations (though I stand to be corrected) was an elevated heart rate in some of the experimental inductions. Zeroing in on the changes that precede abortion in this syndrome should be worthwhile, and if we could do so noninvasively with ultrasound, that could be very rewarding.

Some of the questions that have cropped up during the past two days should be answerable with relatively modest further effort. The weather data that are being amassed, for example, sound very promising for resolving the climatic background to MRLS and, in my view, should continue to be collected and analyzed. Like many others, I’m sure, I am intrigued by the unilateral uveitis found in MRLS. I must say I wonder why nobody seems to have simply passed caterpillars across some experimental eyes to see whether irritation is induced and leads to those changes. I thought it remarkable that there was no such eye effect, nor any pericarditis, in any of the experimental models that I heard about. Dr. Kronfeld’s doubts about whether horses eat caterpillars should surely be relatively easy to confirm or refute. Dr. Kronfeld also underlined the need for a more generalized mycotoxin test; a nonspecific test would be very useful as a screening method to apply before looking for specific mycotoxins. Another relatively simple approach to investigating MRLS might be to look at the allergenic effects of caterpillars.

The call for a laboratory animal model system in which to study MRLS is urgent because of the cost of working with mares and the logistics of assembling enough mares to arrive at useful conclusions. While I agree entirely that we need a laboratory animal model, I would urge you not to get too diverted from the horse as the model of most direct concern because we need to remember the perennial problem of species specificity. You can prove all you like about such-and-such an agent doing such-and-such a thing in such-and-such an animal, but this will not necessarily be true in another species. Dr. Schlafer made the very important point that we are going to need to get at the maternal side of the placenta to go forward. Thus, we are going to need to sacrifice some affected animals; those affected animals, I would suggest, are going to have to come from some kind of reproducible model system. Another

exclusive opportunity that would be provided by a model of MRLS in horses directly would be that of facilitating the frequent blood sampling that is necessary to work out whether or not a bacteremia is associated with the syndrome. That question came up time and time again. If we had a treatment that we knew was going to be causing abortion in mares, there should be no limit to the effort that can go into monitoring them, if the money and the incentive are there. Thus, in my opinion, the work being done directly with horses should and must continue.

As to a laboratory animal model, immune-deficient mice would seem to have great potential (3). I can say from personal experience that growing trophoblast and endometrium together in such mice works beautifully. And so the right target tissue (e.g., placental tissue at a particular stage?) could be used and subjected to various treatments in these mice, making it a lot easier to achieve the kinds of statistics that we really need for MRLS investigation. Choosing the right tissue, of course, remains the \$64,000 question.

There are, needless to say, some much more difficult and more long-term questions that I think we have to address. If Bushy and Bagot are involved, they have to be fractionated, and we have to find out which parts of them are involved. There has been much discussion about that. Drs. Webb and McDowell presented a very nice list of the steps that need to be taken to decide whether a putative agent is biological, environmental, or toxicological, and this approach has to be pursued.

The portal of putative entry is second to none in deciding whether we're talking about an agent that is hematogenous or not. Furthermore, it has been pointed out to me during the workshop that the possibility of a pulmonary route of entry being important received scant attention during our discussions.

In discussing control measures, Steve Johnson of Margaux Farm said that entomologists are now his "new best friends." I don't suppose that was so before this whole problem came up, and I take this as yet another example of the collaboration engendered by the investigation of MRLS.

I was struck by the significance of Dr. Volkmann asking Dr. Rieske-Kinney in which direction her ETC were migrating, experimentally. Coming from Cornell, he was obviously disturbed to think that they were going in a northerly direction; coming from Canada, I know how he feels!

I took Dr. Fitzgerald's mention of laser guns very seriously indeed. That struck me as an excellent idea, and I don't think it should be discarded because I think that the ecological consequences of wiping out cherry trees in this beautiful state would be catastrophic.

And finally, in this field of control measures, Wayne Long in the preceding session put his finger on the real nub of the question as far as the public is concerned: the paramount need to be able to identify safe pastures.

References

1. Goldstein, M. H.; Bazer, F. W.; Barron, D. H. Characterization of changes in volume, osmolarity and electrolyte composition of porcine fetal fluids during gestation. *Biology of Reproduction*. 1980; 22(5):1168-1180.
2. Arthur, G. H. The fetal fluids of domestic animals. In: Perry, J. S., editor. *Water Metabolism in Pregnancy: Proceedings of the Fifth Symposium for the Study of Fertility*; Newcastle upon Tyne. Oxford: Blackwell Scientific Publications; 1969: 45-52. (*Journal of Reproduction and Fertility*. Supplement no. 9).
3. Croy, B. A.; Linder, K. E.; Yager, J. A. Primer for non-immunologists on immune-deficient mice and their applications in research. *Comparative Medicine*. 2001; 51(4):300-313.