

**Title:** Expanding the immune toolkit for assessing pig health and improving swine disease and vaccine studies NPB project [NPB# 06-043 renewal of # 05-015]

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(with major collaboration with Dr. Serge Muyldermans, Free Univ. Brussels, Belgium)

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### Industry Summary:

Keeping pigs healthy and productive is a major goal for producers and researchers. Preventing disease, using biosecurity and planned vaccinations, are hallmarks of well-managed swine facilities. In the case of disease outbreaks, diagnostic laboratories use an array of tests to quickly identify underlying infections and causative pathogens; once diagnosed veterinarians can prescribe therapies to treat the infections and propose vaccinations to prevent disease outbreaks in other pigs. All of these disease associated functions require reagents that can quantitate the infection and identify and quantitate swine immune responses, specifically serum antibodies or immunoglobulins (Igs) that affirm pathogen exposure. Additionally most vaccine programs require anti-swine Ig reagents to detect IgG antibody levels that confirm the success of virus eradication programs. In mammals there are numerous IgG subclasses, which have different activities. Currently most investigators rely on polyclonal antisera, prepared typically in goats or rabbits, to identify swine IgGs. However these polyclonal antisera are tedious to prepare, lack immortality, vary between batches, and usually exhibit reactivity for multiple swine IgG subclasses. Thus we proposed to produce mouse monoclonal antibody (mAb) reagents made by hybridoma technology. MAbs recognize only one epitope, thus the name monoclonal. It is generally stated that a mAb can “recognize a needle in a haystack” whereas a polyclonal recognizes “the needle and the haystack”. Furthermore, mAb provide a sustainable and renewable resource, so data from laboratories around the world can be compared. Our goal was to address swine reagent needs by producing mAb reagents that uniquely identified each IgG subclass for more effective swine immunity quantitation.

Our first objective was to “Identify all immunoglobulin-G (IgG) subclass genes; express Ig proteins for each swine IgG subclass gene.” This was completed using molecular genetic approaches at the Univ. of IA. All IgG subclass cDNAs were cloned and sequenced. These clones were then provided to our collaborator in Belgium to express quantities of each specific IgG protein. This was performed using their camelid-porcine Ig expression system. The expressed proteins prepared in Belgium were shipped to the US where they were used to address objectives B “Characterize the reactivity of known anti-swine Ig monoclonal antibody (mAb) reagents with each Ig gene product” and C “Develop new mAbs that are specific for each of the expressed IgG subclass proteins.”

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For Obj. B previously developed hybridoma cell lines expressing mAb reactive with swine Igs were collected at BARC from labs worldwide. Hybridoma supernatants containing mAb were prepared, purified and sent to Iowa for use in their tests of specific reactivity with the expressed swine IgG subclass. As expected none of the currently available anti-swine IgG mAbs was specific for just one IgG subclass. Thus Obj. C had to be pursued. Mice were separately immunized with each swine IgG subclass protein, as specific camelid-porcine IgG. All hybridoma fusion supernatant mAb produced were tested for their reactivity with swine Igs and the camelid-swine IgG subclass proteins. Several fusions were performed but unfortunately to date no mAb has been developed that reacted against just one swine IgG subclass protein. [It should be noted that as part of a separate funding initiative these immunizations and hybridoma fusions are continuing.] Comparative analyses of ELISA data for every new mAb were performed, and compared to known standards and control IgGs.

The final objective was “Distribute mAbs and develop reference standard sera.” We worked with the USDA Animal and Plant Health Inspection Service (APHIS) National Veterinary Services Laboratories (NVSL) facilities in Ames IA to establish a resource for veterinary reagents; NVSL has made two anti-swine reagents, anti-IgM (M160) and anti- IgA (1459), available to researchers. It is hoped that this will be just the first step in NVSL making a broader panel of immune reagents available to veterinary researchers. The development of the USDA APHIS NVSL resource is a major accomplishment for this grant.

In summary, researchers require IgG subclass specific reagents to determine Ig function; diagnostic laboratories use them to measure Ig levels and specific antibody responses. This NPB project (#06-043 and #05-015) attempted to develop a full panel of well-characterized new mAb reagents that uniquely recognized swine each IgG subclass protein. It affirmed the reactivity of previously produced mAb but was unsuccessful at producing new mAb reagents. Once produced such mAb reagents will refine swine disease diagnostic tests and enable scientists to more accurately compare results among labs, thus opening up new understanding of disease control mechanisms and pathologies, as well as better characterizing swine vaccine responses.

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**Keywords:** include at least 5 keywords

Swine antibody, immunoglobulin-G, IgG, monoclonal antibody, immune reagent; vaccine response; serum diagnostic reagents

**Scientific Abstract:**

Swine disease and vaccine research has been advanced by the development of sophisticated tools to measure physiologic parameters associated with immunity, pathology, and disease prevention. Our goal is to expand the immune toolkit for pigs, by developing and characterizing reagents that can be used to identify and quantify a major class of immune proteins, the antibodies or immunoglobulins (Igs) that swine produce in response to infection or vaccination. Scientists measure pathogen exposure and vaccine efficacy by quantitating Ig levels in serum. But not all Igs are equal. We know for porcine reproductive and respiratory syndrome virus (PRRSV) infections that there is a well-characterized antibody response as measured by the IDEXX ELISA. Neutralizing Igs are known to take longer to develop but are important in recovery from PRRSV infection. It is likely that these sets of Igs represent different IgG subclasses. Currently most investigators rely on polyclonal antisera that are tedious to prepare, lack immortality, are usually not class specific, and vary between batches. For this NPB project we characterized known, and attempted to develop new, monoclonal antibody (mAb) reagents that uniquely recognize the various swine IgG subclasses.

Our first objective was to “Identify all immunoglobulin-G (IgG) subclass genes; express Ig proteins for each swine IgG subclass gene.” We cloned genes from the previously known 5 swine IgG genes. Research at Dr. Butler’s lab at the Univ. of IA actually resulted in the discovery of numerous other swine IgG genes, thus adding

additional complexity to our goals. We worked with a collaborator, Dr. Serge Muyldermans, in Belgium to express the original 5 known swine IgG subclass proteins in vitro using his novel camelid-swine Ig expression system that efficiently produces single chain porcine-camelid chimeric IgGs. With this camelid system we have the means to express only constant regions of each specific swine IgG heavy chain protein and to use each porcine-camelid chimeric IgG to immunize mice and to produce and characterize mAb. The expressed proteins prepared in Belgium were shipped to the US where they were used to address objectives B “Characterize the reactivity of known anti-swine Ig monoclonal antibody (mAb) reagents with each Ig gene product” and C “Develop new mAbs that are specific for each of the expressed IgG subclass proteins.” For Obj. B previously developed hybridoma cell lines expressing mAb reactive with swine Igs were collected at BARC from labs worldwide, including anti-IgG hybridomas from the UK and anti-swine IgA and IgM hybridomas from Canada. Hybridoma supernatants containing mAb were prepared, purified at BARC and sent to Iowa for use in their tests of specific reactivity with the expressed swine IgG subclass. As expected none of the currently available anti-swine IgG mAbs was specific for just one IgG subclass. Thus Obj. C had to be pursued.

We first concentrated our efforts on what research had affirmed are the most highly expressed swine IgGs: IgG1, IgG3 and IgG5. We targeted a broadly reactive anti-IgG1, the major IgG in the blood of pigs; currently we have anti-allotypic mAb only. We targeted IgG3, the most important IgG for Complement fixation as well as the major IgG in the intestinal tissues of the newborn piglet. Finally we tried to make mAb to IgG5, the third most expressed IgG in the pig. For Obj. C we used the Hybridoma Facilities at Iowa State Univ. for hybridoma production. Mice were separately immunized with each swine IgG subclass protein, as the specific camelid-porcine IgG. All hybridoma fusion supernatant mAb produced were tested for their reactivity with swine Igs and the camelid-swine IgG subclass proteins. Several fusions were performed but unfortunately to date no mAb has been developed that reacted against just one swine IgG subclass protein. New fusion efforts have been put on hold waiting for new supplies of porcine-camelid chimeric IgG construct proteins to continue this immunization work. [It should be noted that as part of a separate funding initiative these immunizations and hybridoma fusions are continuing.]

The final objective was “Distribute mAbs and develop reference standard sera.” We worked with the USDA Animal and Plant Health Inspection Service (APHIS) National Veterinary Services Laboratories (NVSL) facilities in Ames IA to establish a resource for veterinary reagents; NVSL has made two anti-swine reagents, anti-IgM (M160) and anti- IgA (1459), available to researchers. It is hoped that this will be just the first step in NVSL making a broader panel of immune reagents available to veterinary researchers. The development of the USDA APHIS NVSL resource is a major accomplishment for this grant. Our overall goal has been to have a full panel of well-characterized mAb that react specifically with each swine Igs to expand our understanding of disease control mechanisms, immunity and pathology.

## **Introduction:**

Our goal has been to develop a broad panel of reagents that identify swine antibodies, specifically the swine immunoglobulin-G (IgG) subclass proteins. It is clear from studies in species like cattle, mice and humans that the nature of IgG subclass responses provide valuable clues as to how the immune system is responding to a particular pathogen or to a vaccine and what protective Igs are important for maternal protective immunity passed from the sow. The reason why this information is needed is because different subclasses of IgG have different effector functions in protecting pigs from infections.

Our goal has been to provide researchers and diagnostic laboratories the required reagents with proven and well-defined pig IgG subclass specificity to make these measurements. To do this for swine meant first identifying all the candidate subclass IgGs. Past and current work has affirmed that pigs have many different expressed IgG subclasses. Based on Univ. of IA studies swine IgG1 is the major IgG1 expressed in the blood of pigs, so it is important that a reliable anti-IgG1 mAb be available. IgG3 is the ancestral porcine IgG which, based on comparison of sequence motifs, should be most important swine IgG for C fixation and Fc receptor binding. Swine IgG3 is the major IgG in the intestinal tissues such as the mesenteric lymph nodes and ileal Peyer’s Patches of the newborn piglet. It is currently hypothesized that swine IgG3 is part of a “first responder”

mucosal immune response for the newborn pig (J.E. Butler, unpublished). IgG5 is the third most expressed swine IgG, at least in newborn pigs. Thus these NPB funded studies have especially been focused on reagents that can be used to collect reliable data on antibody responses from these major IgG subclasses and to truly quantitate their concentrations in various body fluids.

Importantly, for making IgG specific reagents, these IgG subclass proteins cannot be separated and purified biochemically, by protein A or G columns, or other chromatographic techniques. Thus, efforts had to be made to develop alternate separation or expression technologies to prepare swine IgG subclass reagents. Once these are available, information on specific IgG responses can be collected and used in the formulation of new diagnostic tests, therapeutics and vaccines with the goal of producing those IgG subclass responses that are most effective and that best mirror the protective response of the pig to natural infections.

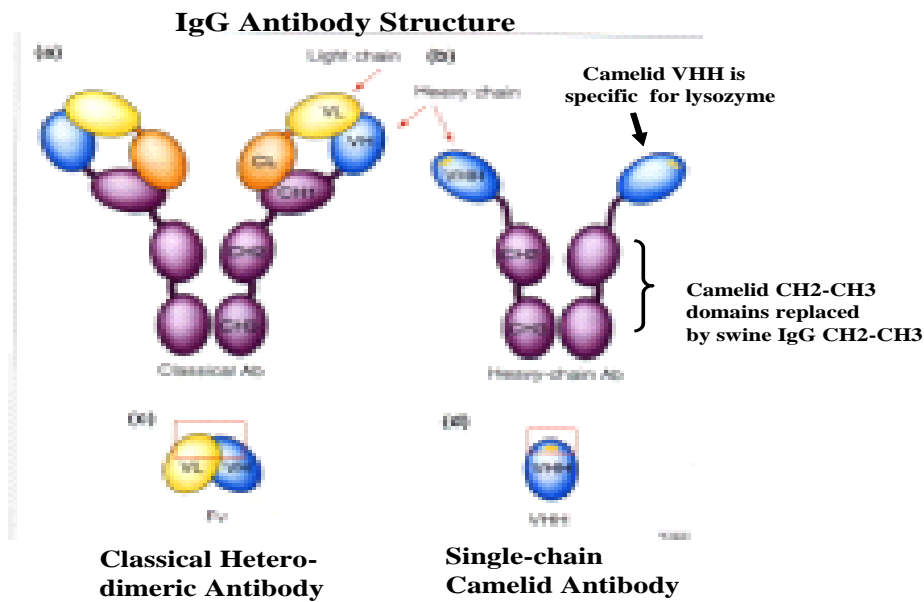
**Objectives:**

- A. *Identify all immunoglobulin-G (IgG) subclass genes; express Ig proteins for each swine IgG subclass gene.*
- B. *Characterize the reactivity of known anti-swine Ig monoclonal antibody (mAb) reagents with each Ig gene product.*
- C. *Develop new mAbs that are specific for each of the expressed IgG subclass proteins.*
- D. *Distribute mAbs and develop reference standard sera*

**Materials & Methods:**

- A. *Identify all IgG subclass genes; express Ig proteins for each swine IgG subclass gene. Prepare Ig proteins as standard references.*

By the end of the grant all swine IgG subclass cDNAs were prepared at Univ. IA and clones sent to Belgium where they were inserted into the appropriate camelid-swine Ig vector. Specifically once all expressed swine IgGs were sequenced and identified, our efforts with our collaborator Dr. Serge Muyldermans of Belgium were aimed at generating chimeric Heavy chain only antibodies (HCABs) consisting of a camelid VHH domain, and porcine hinge region, and CH2 and CH3 domains of the different porcine IgG subclasses and allotypic variants (Fig.1) (1). Thus, major parts of each swine IgG constant region were cloned into a vector and expressed with the camel VHH as single heavy chain porcine-camelid chimeric IgG (HCABs). These chimeric HCABs could then be used (a) to generate subclass-specific monoclonal antibodies and (b) as golden standards for determining the specificity of existing polyclonal and monoclonal antibodies to porcine IgG. The IgG subclass proteins were expressed in Belgium and shipped to the US.



**Fig.1. Comparison of swine “classical” heterodimeric IgG heavy and light chain structure with “single chain” or camelid Ig structure**, noted as Heavy chain only antibodies (HCABs). Note: area for substitution of partial swine IgG heavy chain sequences (CH2-CH3) in combination with known lysozyme specific binding region (VHH) (3).

*B. Characterize the reactivity of known anti-swine Ig mAb reagents with each Ig gene product.*

Previously developed hybridoma cell lines expressing mAb reactive with swine Igs were collected at BARC from labs worldwide. Hybridoma supernatants containing mAb were prepared and mAb purified for tests of IgG subclass specificity. All mAb will be tested for their reactivity with swine Igs and camelid-swine IgG subclass proteins using ELISA technology.

*C. Develop new mAbs that are specific for each of the expressed IgG subclass proteins.*

Mouse that immunizations and fusions for every swine IgG subclass protein have been attempted. It is hoped that mAb will be developed against each swine IgG subclass protein. Comparative analyses of ELISA data for every new mAb, as well as previously characterized mAb, will be performed at Univ. IA and BARC. mAb reactivity will be tested against each swine IgG subclass protein and camelid-swine IgG subclass protein and compared to known standards and control Igs. Appropriate statistical analyses will be used to affirm specificity and sensitivity.

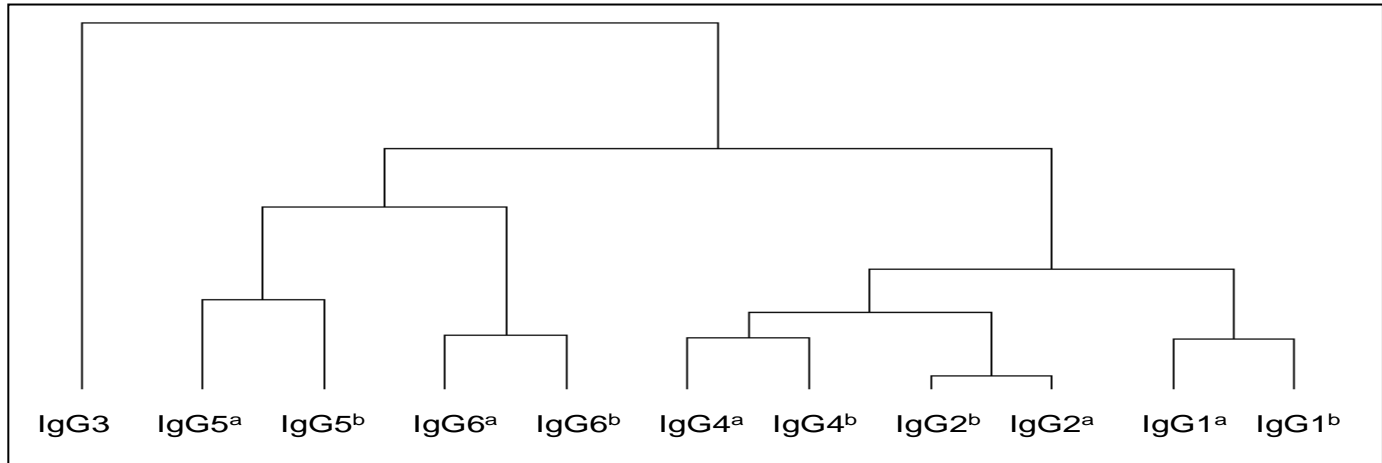
**Results:**

*A. Identify all IgG subclass genes; express Ig proteins for each swine IgG subclass gene.*

*A1. Identify and clone expressed swine IgGs*

Porcine IgG subclass proteins cannot be separated and purified by biochemical methods but their genes have been cloned at Dr. Butler’s lab at Univ. IA. During the course of these NPB grant studies the original assumptions of the number of swine IgG genes have been expanded. It is now clear that one can identify 11

total, and 6 functional, IgG subclass genes in the swine genome (Fig. 1). This is documented in Dr. Butler's recent publication which acknowledges NPB funding (2).



**Figure 2. Swine IgG heavy chain gene sequence alignment dendrogram (2, 3).**

Molecular studies at Univ. IA have verified that swine have two light chain types, kappa (IgL $\kappa$ ) and lambda (IgL $\lambda$ ), one known heavy chain gene each for IgM, IgA, IgD and IgE, but multiple IgG heavy chain subclass genes (Fig. 2) (2, 3). All of the 11 swine IgG genes have been sequenced and their relative homology to other swine IgGs assessed using statistical tools. The Figure 2 dendrogram shows the relative sequence similarity among the 11 swine IgG heavy chain genes; the larger the distance between each gene the greater the sequence difference. Each major branch in the Fig.2 dendrogram identifies the product of one functional gene, i.e. subclasses IgG1 through IgG6. Each minor branch identifies two genetic variants (alleles) of each swine IgG subclass gene found for all swine IgGs except IgG3. In addition to these functional IgG genes, recent mapping of the heavy chain genome of swine (4; Eguchi-Ogawa, Uenishi, Butler, unpublished data) shows that swine also have several non-functional IgG genes, which are referred to as pseudogenes.

## ***A2. Updates on protocols for production of camelid – porcine HCABs***

Once all expressed swine IgGs were sequenced and identified, our efforts with our collaborator Dr. Serge Muyldermans of Belgium were aimed at generating chimeric Heavy chain only antibodies (HCABs) consisting of a camelid VHH domain, and porcine hinge region, and CH2 and CH3 domains of the different porcine IgG subclasses and allotypic variants (Fig.1) (1, 5). Thus, major parts of each swine IgG constant region were cloned into a vector and expressed with the camel VHH as single heavy chain porcine-camelid chimeric IgG (HCABs). These chimeric HCABs could then be used (a) to generate subclass-specific mAbs and (b) as golden standards for determining the specificity of existing polyclonal and monoclonal antibodies to porcine IgG.

During these NPB grants such chimeric HCABs were prepared in Belgium by constructing specific camelid-swine IgG plasmids and using them to transfect mammalian NSO cell lines. Once established the expressed proteins were tested for camelid-swine IgG. However two problems were encountered: First, the recombinant chimeric HCABs were difficult to purify specifically on Protein A or Protein G because the IgG from Fetal Calf Serum (FCS, added as a supplement to culture the transgenic NSO cell lines) co-purified with the chimeric HCABs. Trials to grow the cells in FCS depleted of bovine IgG failed (or gave yields that were too low to be practical). Generating new transgenic cell lines in CHO-S cells (cells that are adapted to synthetic culture medium without FCS) yielded even lower amounts of chimeric HCABs. Secondly, attempts were made to purify the chimeric HCABs by affinity adsorption on chicken egg-white lysozyme, the antigen recognized by the camelid VHH. However, the binding was so strong that the chimeric protein bound irreversibly and could not be

eluted. A switch to another VHH with a faster Koff (faster dissociation from lysozyme) but this didn't solve the problems.

Consequently the Belgian group decided to adapt a new strategy in 2009. All the pig IgG CH2 and CH3 domains were recloned behind a new camelid VHH. The new VHH binds strongly to chicken egg-white lysozyme and only very weakly to turkey egg white lysozyme. This development meant that after recloning the transgenic cell lines that grow well in rich (FCS containing) medium could be generated and good quantities of expressed proteins collected. Those HCABs could then be purified using affinity chromatography on turkey lysozyme. This will yield reproducible batches of the chimeric camelid-swine IgG HCABs. Unfortunately, turkey lysozyme is no longer commercially available; it had to be prepared from fresh eggs. After checking its purity a turkey lysozyme affinity adsorbent was made with the MicroLink Protein Coupling kit. Various single domain VHH were purified and tested to identify a VHH that binds very weakly to turkey lysozyme and strongly to chicken lysozyme; clone D2L19 has been identified as the appropriate VHH for the camelid-swine IgG HCABs. When needed for mAb production screening in ELISA the chimeric HCABs can be coated on the plate via chicken lysozyme as antigen.

Due to these major changes in procedures new camelid-swine IgG constructs planned for 2009 are still in process of being finalized. It is projected that a full panel of camelid-swine subclass IgG products will be available in 2010. Plans for the continued production and testing of camelid-swine IgG and development of mAb were incorporated into the recently funded renewal of the USDA NIFA grant #2006-35204-16880 for the U.S. Veterinary Immune Reagent Network (US VIRN) [www.vetimm.org](http://www.vetimm.org).

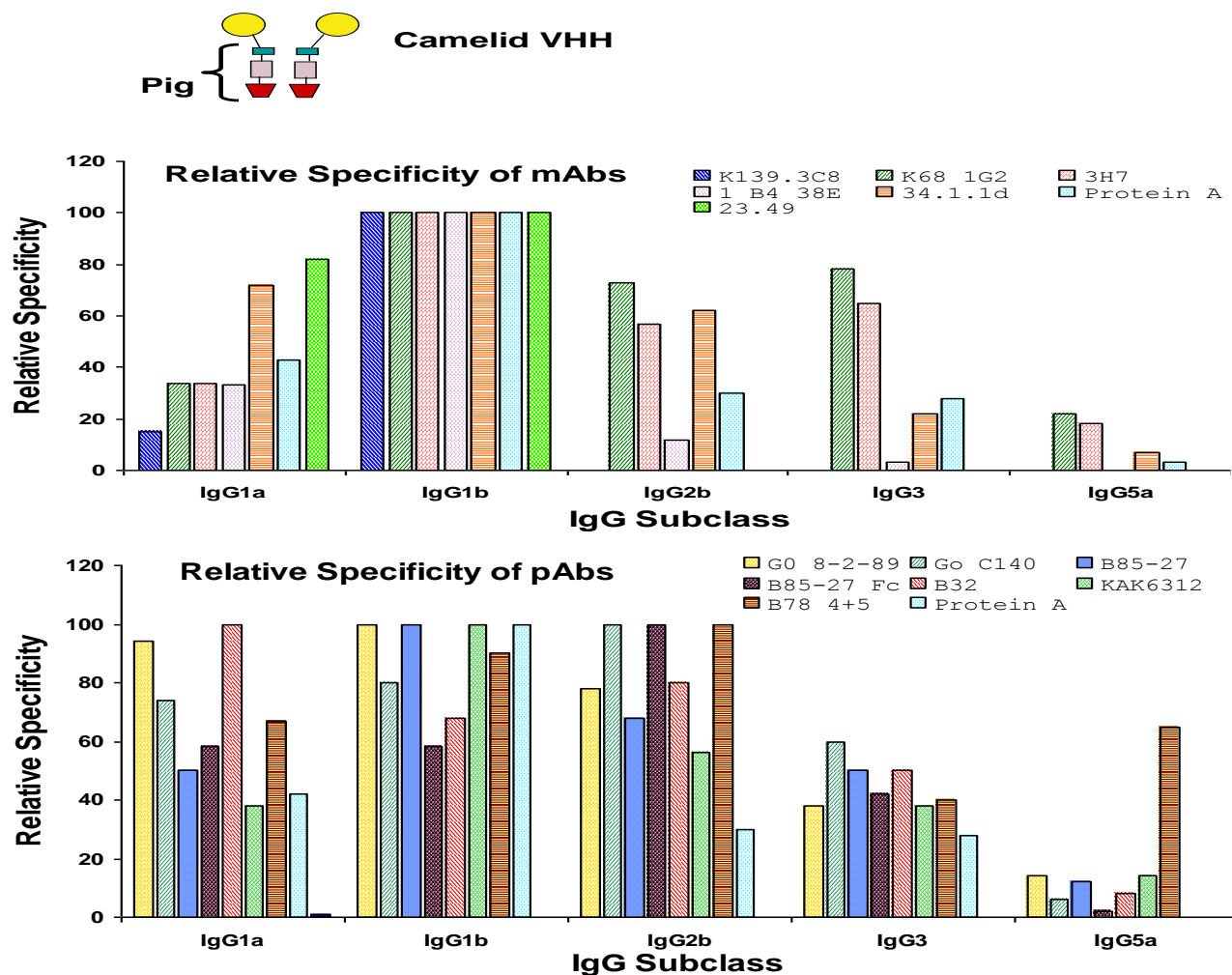
### ***A3. Identify priority targets for production of camelid – porcine HCABs***

The overall plan is to target the IgG genes and their alleles that are most frequently used in the antibody responses of swine express in tissue culture using the in vitro camelid-swine Ig expression system. For swine these are the alleles of IgG1, IgG5 and IgG3 (2, 3). Using the original expression system low amounts of purified IgG proteins of each subclass and allotype were produced. These expressed proteins had a tight binding specificity for lysozyme that was used for ELISA work (see Fig.3 below) for functional studies of swine IgG subclasses and binding tests on the specificity of existing mAb to swine IgG (Fig. 3).

The first generation of chimeric swine IgG HCAB proteins was used in specificity testing (Fig. 3). These batches were found to be contaminated with proteins from the FCS culture media in which they were grown. This did not seriously interfere with the specificity testing but rather with using these protein preparations to make mAbs (See Section C). Thus the procedure was re-designed by Dr. Muyltermans, as discussed above.

### ***B. Characterize the reactivity of known anti-swine Ig mAb reagents with each camelid – porcine HCAB***

The first generation of HCAB proteins for 5 swine IgG subclasses or their allelic variants were tested for reactivity with currently available anti-swine IgG mAbs and a spectrum of polyclonal antibodies (pAbs) anti-swine IgG. Fig. 3 shows the results of an ELISA that bound each HCAB to lysozyme and then asked whether previously characterized mAb and pAb were reactive with one or more camelid – swine HCAB.



**Fig. 3. Reactivity of panel of monoclonal antibodies (mAbs) (top) and polyclonal antibodies (pAbs) (bottom) antibodies with the first generation of expressed camelid-swine IgG proteins (HCAb). The legend identifies the different camelid-swine HCAs that were bound to the lysozyme plate and the mAbs and pAbs that were tested for reactivity by ELISA.**

Most currently available anti-swine IgG mAbs recognized more than one subclass IgG or their allelic variant based on HCAb ELISA binding results (Fig.3). One mAb, K139.3C8, appears to be among the most specific. Unfortunately its specificity is for the IgG1b, a specific allelic variant of IgG1; this mAb did not bind to IgG1 subclass proteins as a whole. This allele, not subclass, specificity phenomenon is not uncommon with mAbs. Without verification and correction this apparent subclass specificity could have serious consequences. For example, if data on antibody response in swine were measured using the apparent IgG1 specificity of K139.3C8, a pig that is homozygous for the IgG1a allele would appear as having no IgG1 immune response because a biased reagent was used for detection. This problem is now being corrected. A different issue is mAb which react with more than 1 swine IgG subclass. The mAbs K68.162 and mAb 23.4, which has been referred to as IgG2-specific in published data, recognize IgG2b, but also IgG3 and IgG1b. MAb 34-1-1 has much broader reactivity being strongly reactive with IgG1a, IgG1b, IgG2b, and weaker with IgG3 and IgG5. These studies are continuing as new mAb are produced and tested.

Most pAbs behave broadly, i.e. they recognize all swine IgGs, except for IgG5, based on ELISA reactivity with the first generation chimeric swine IgG proteins (Fig. 3, bottom). We included the characterization of pAbs since these are the antibodies most frequently used by investigators and diagnostic labs. This study was not exhaustive and could be expanded to include more commercially available reagents;

however, the problem with pAb is that every preparation can have different specificity and thus one would have to screen every commercial lot to be sure of their exact reactivity.

**C. *Develop new mAbs that are specific for each of the expressed IgG subclass proteins.***

For the NPB project we planned to characterize porcine IgGs and then use them to perform immunizations and hybridoma fusions to develop new mAbs reagents that uniquely recognize the various Ig isotypes and IgG subclasses. We had planned to use the Univ. Iowa Hybridoma Facilities; however, those facilities were closed in Feb. 2007. As a consequence of this unexpected change, a new hybridoma group had to be identified. A working relationship with Paul Kapke at the Hybridoma Facility at Iowa State University (ISU) [http://www.biotech.iastate.edu/service\\_facilities/hybridoma.html](http://www.biotech.iastate.edu/service_facilities/hybridoma.html), that performs hybridoma fusions and produces mAbs for many investigators at ISU and other universities in the Midwest, had to be established. Unfortunately, ISU had limited experience in preparing mAbs to immunoglobulins. In 2009, Paul Kapke and Dr. Butler worked together to improve immunization and screening procedures to start to develop the first new generation of subclass-specific anti IgG mAb for swine. This project has now generated several hybridomas that appear to produce IgG subclass specific mAbs. These have the potential to solve the biased allotype problem. Despite the ending of this grant, plans have been affirmed to perform several more fusions at ISU. Additional fusions will be funded by the renewal of the USDA NIFA US VIRN grant.

**D. *Distribute mAbs and develop reference standard sera***

We have worked with the USDA APHIS NVSL facilities in Ames IA to establish a resource for reagents. The Center for Veterinary Biologics (CVB) of NVSL has made two anti-swine reagents, anti-IgM (M160) and anti- IgA (1459), available to researchers at the cost of shipment. It is hoped that this will be just the first step by the CVB in making a broader panel of immune reagents available to veterinary researchers.

**Discussion:**

During the last 5 years the study of swine IgG subclasses has been reinvestigated at the genetic level. These revealed major discoveries, multiple swine IgG C gamma genes, some appearing to be the result of recent gene duplications in domesticated swine, as well as a large number of allelic variants of each IgG C gamma gene (Figure 2). Thus the swine IgG system could be the most polygenic and polymorphic antibody system ever described (2, 3). With the availability of the sequences of more IgG genes (Fig. 1) in silico analyses can be performed. Some modeling can now be done that could be predictive of the biological function of each encoded swine IgG subclass, by comparison to the structure of human IgG subclass proteins for which function is known (3). National and international collaborations have now been established to help resolve this complexity by looking into genomic organization of the porcine heavy chain locus and breed distributions of the major allotypes of swine IgG. Therefore, the work that we originally proposed (to clone and express all swine IgG genes and verify reactivity of known mAb on each of them) has grown more complex, well beyond our early estimates.

Because more swine IgG genes were discovered more expressed proteins had to be produced. The sequences for each of the new genes first had to be finalized. This is now completed and all expressed swine IgG subclass have been provided as cDNA by Dr. Butler to Dr. Muyldermans. In Belgium new protocols have had to be established for the efforts to reclone the swine IgG genes into the updated camelid expression vector and then expressed as a new IgG HCAb. These efforts are still underway based on committed funding. Due to the unexpected closure of the Univ. Iowa Hybridoma Facilities in Feb. 2007, and concern for contamination of first generation chimeric HCAb, hybridoma production plans were placed on hold until new porcine-camelid chimeric HCAb were produced based on the updated protocol described above. These efforts are still underway

based on committed funding and further support through the USDA NIFA US VIRN grant. The development of the USDA APHIS NVSL resource is a major accomplishment for this grant. We can now assure researchers that mAb M1459 does not distinguish between IgA allotypes when tested along with other reagents (10).

The preparation of reagents for veterinary research does not take place in a vacuum or is it necessarily restricted to the objectives of an original research plan. One must adapt and alter objectives as is dictated by new incoming data. Parallel research can contribute to achieving an objective, even though not originally listed as an objective. Several studies fit into this category and will likely help to clarify issues regarding neonatal immunity. First, problems with reagent specificity are not restricted to the IgG subclasses; a case in point is porcine IgA which occurs in two allelic forms. The first anti-IgA mAbs gave disparate results suggesting there were two subclasses of IgA in swine (7). With the characterization of the gene encoding IgA in swine (8, 9) research revealed that the original mAbs to porcine IgA really distinguished the two IgA allotypes. This warranted a study of the specificity of other available anti IgA mAbs, the results of which are now published as part of a study on the swine response to vaccination or natural infection with foot and mouth disease virus (10).

The confirmation of the data obtained on mAb made to porcine IgGs and other Igs depends on completion of mapping of the porcine IgG heavy chain genome. Although not originally proposed as an objective, these ongoing studies have resulted in one publication (4) and a planned second publication. First, Eguchi-Ogawa et al (4) showed that, in the map of the porcine IgG heavy chain genome, IgG3 is coded by the most 3' *IGHG* gene, *IGHG3*, and it is followed by *IGHG5*. These two genes encode one-third of the swine repertoire and both are located as the most 5' *IGHG* genes. Why does this matter? It is very likely that in fetal and newborn piglets, IgG3 and IgG5 are expressed early and play a major role as "natural antibodies" in protecting the neonate from infections.

Our overall goal is to have a full panel of well-characterized mAb that react specifically with each swine Ig isotype and IgG subclass so scientists will be able to compare accurately the Ig functions. Once developed, these mAb reagents will refine swine disease diagnostic tests and enable scientists to more accurately compare results among labs. We expect that use of such mAbs will reveal details of the functions of each swine Ig isotype and subclass, thus opening up new understanding of disease control mechanisms and pathologies, as well as serve as improved tools for characterizing swine vaccine responses.

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