Recombinant Antibodies in Veterinary Medicine: An Update

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The production of recombinant antibodies has had a tremendous impact on several research fields, most prominently in biotechnology, immunology and medicine, enabling enormous advances in each. Thus far, a broad diversity of recombinant antibody (rAb) forms have been designed and expressed using different expression systems. Even though the majority of rAbs approved for clinical use are targeted to humans, advances in veterinary medicine seem promising. The aim of this mini-review is to present an update regarding the rAbs in veterinary medicine reported to date, as well as their potential use in diagnostics, prophylaxis and therapeutics. Full- and single-chain fragment variables are the most common forms of rAbs developed for the detection, prevention and control of parasitic, bacterial and viral diseases, as well as pain and cancer treatment. Nonetheless, advances in research seem to be skewed toward economically important animals, such as pigs, cows, poultry and dogs. Although significant results have been obtained from the rAbs reported here, most have not been developed enough to be approved. Further research and clinical trials should be encouraged to enable important findings to fulfill their intended potential to improve animal well-being.

Keywords: recombinant antibodies, biotechnology, veterinary medicine, single-chain antibodies, nanobodies, chimeric antibodies

INTRODUCTION

Biotechnology has allowed for alternative methods of antibody (Ab) production, thereby reducing or eliminating the use of experimental animals (1). The selection of an expression system, such as bacteria, yeast, insect and mammalian cell lines, and transgenic plants (2, 3), depends primarily on the type of Ab desired. Frenzel et al. (4) published an excellent review on the expression of recombinant antibodies, discussing the pros and cons of the most-used systems (4).

Abs are glycoproteins that consist of two heavy (H) and two light (L) chains united by disulfide bonds. Light and heavy chains have a variable (V) and a constant (C) region; in turn, the constant heavy domain (CH) is divided into three domains (CH1-CH3) and in some cases, four domains. Variable regions contain “complementarity determining regions” (CDR) that determine the affinity and specificity of an Ab. The union of variable domain of light chain-constant domain of light chain (VL-CL) and variable domain of heavy chain-constant domain of heavy chain (VH-CH1) form the “antigen-binding fragment” (Fab). The rest of the Ab forms the fragment crystallizable region (Fc) and gives the Ab its effector function (5). The above characteristics represent the classical structure of an Ab.

Based on the classical structure of an Ab, other forms of rAb have been developed (Figure 1). The most popular in veterinary medicine seems to be the single-chain fragment variable (scFv). The scFv comprises the VL-CH1 joined by a short peptide linker (6). Another highly reported rAb is the single-domain antibody (sdAb) or nanobodies, which present the heavy variable region (VHH) only (7).
Other rAbs, such as triabodies and tetrabodies (2), are not particularly reported in veterinary medicine (Figure 1). Hybrid rAbs, chimeric or “–nized,” are common in veterinary medicine. A chimeric Ab typically consists of the V regions of one Ab and the C region of another. The “–nized” Ab comprises the original Ab and CDRs from other species, such as humanized or porcimized. The main advantage of a hybrid rAb is reduced immunogenicity while maintaining the specificity of CDRs (8).

Orthoclone (Muronomab-CD3) was the first monoclonal Ab (mAb) approved in humans (9). Since then, Abs have become predominant products in the human pharmaceutical market (10). Since 2015, 23% of the drugs approved by the Food and Drug Administration in USA have been Abs, including humanized and chimeric, with just a few used in animals. This review presents an update of the rAbs reported in the field of veterinary medicine according to their use in diagnosis, prophylaxis and therapeutics in different species. However, there is an emphasis on rAbs in pigs and cows because they represent more prolific fields. Table 1 summarizes all rAbs described in this mini-review, showing the expression system, form, target species and main results. A discussion of the results describing only rAb production was omitted in the following sections.

RECOMBINANT ANTIBODIES IN VETERINARY DIAGNOSIS

Pig

Research for new diagnostic tests using rAbs have been concentrated on porcine circovirus type II (PVC2), classic swine fever virus (CSF), Brachyspira hyodysenteriae and Taenia spp. A commercial PVC2 vaccine was used to immunize a camel and produce a sdAb anti-Cap protein (11). This sdAb showed high specificity and sensitivity for the detection of PVC2 without cross reactivity with PCV1, porcine reproductive and respiratory syndrome virus (PRRSV) GP5 protein or CSF E2 protein. The sdAb was fused with alkaline phosphatase (sdAb-AP) to improve diagnosis, and the affinity and sensitivity were higher than those of the original sdAb (12). Recently, a porcimized rAb anti-E2 protein of CSFV was produced. This porcimized rAb was evaluated in diverse assays with good results and importantly, retained the ability to neutralize CSFV in vitro, suggesting that it has great potential as a diagnostic tool (13).

Lobova et al. (14) described a scFv capable of detecting B. hyodysenteriae, an etiological agent of swine dysentery. After ELISA and immunofluorescent assay (IFA) evaluation, the authors concluded that this scFv can be used in new diagnostic tests (14). In the case of parasitic diseases, a sdAb showed no cross reactivity with T. saginata, T. hydatigena, T. crassiceps, and Trichinella spiralis antigens, allowing the specific diagnosis of Taenia solium infection (15).

Cows

The rAbs for the most important pathogens affecting bovines, including foot and mouth disease virus (FMDV), Mycobacterium bovis and bovine spongiform encephalopathy (BSE), have been evaluated by new diagnostic tests. Two scFvs specific for the 3ABC antigen have been reported to differentiate between vaccinated and infected animals with FMDV. Foord et al. (16) produced a chicken scFv specific for the 3B region of the 3ABC antigen and concluded that scFv could be used in a FMDV differentiating infected from vaccinated animals (DIVA) test offering superior results compared with those of the 3ABC antigen in an ELISA, using sera from naive and infected animals (16). Sharma et al. (17) validated a scFv in sandwich and competitive ELISAs and proposed it as an alternative to the diagnosis of FMDV (17). Other authors have produced a scFv anti-VP1 protein of FMDV in transgenic Tobacco plants (18). In the case of the bovine immunodeficiency virus (BIV), a scFv anti-capsid protein was produced and showed better sensitivity in ELISA and Western blot (WB) assays than did the mAb, which is considered the “gold standard” (19). A scFv anti-HSP65 protein was conjugated to colloidal gold and evaluated in immunochromatographic tests (ICT) as a capture/secondary antibody to improve M. bovis diagnosis. This combination could detect the HSP65 protein dimer by ICT but not by ELISA. Later, this scFv was fused with a chicken H chain, and the stability was improved without affecting functionality (20). In another study, a sdAb produced in camels immunized with intracellular bacteria Brucella melitensis, which is responsible for Mediterranean fever in animals and humans, could recognize antigens of a B. melitensis in ELISA (21). Finally, recombinant chicken immunoglobulin Y (IgY) (Ab3-15 and Ab4-19) anti-prion protein (PrPSc) was produced to diagnose BSE, showing that it can be used to diagnose BSE and other prion diseases (22).

Others

In poultry, a scFv specific for the avian influenza virus (AIV) was produced and evaluated by ELISA; it showed higher sensitivity and specificity than previously established protocols (23). Additionally, scFv anti-phosphoprotein of the Newcastle disease virus (NDV) showed potential as a detection tool in ELISA and WB (24). A similar scenario was reported for a scFv specific to the infectious bursal disease virus (IBDV) (25, 26) and a scFv specific to avian coccidiosis (27). Taking advantage of the similarities between the human epidermal growth factor receptor 2 (HER2) and its canine homologue protein, dog epidermal growth factor receptor (DER2), two rAbs with cross reactivity were produced, a Fc-sdAb and a GFP-sdAb. Recognition of HER2 and DER2 was evaluated by flow cytometry and IFA to detect breast cancer cells from humans (SKBR3) and dogs (SH1B and P114). Therefore, these rAbs can be used to identify malignant cells and have the potential to be used as immunotherapeutics in dogs and humans (28).

RECOMBINANT ANTIBODIES IN IMMUNOPROPHYLAXIS

The amount of research on rAb as a new immunoprophylaxis for pigs has been enormous compared with that for bovine or other species. Different forms of rAb for porcine epidemic diarrhea virus (PEDV), PRRSV, FMDV, African swine fever virus (ASFV), and Haemophilus parasuis have been produced and evaluated.
However, the majority of rAbs have been directed to PRRSV control.

**Pigs**

Antigen targeting to antigen-presenting cells, such as dendritic cells (DCs), has become an attractive approach in veterinary medicine (67). Other authors have evaluated this strategy but not with rAb (68–70). Subramaniam et al. has probed a scFv-Fc (mouse x pig) specific to DC-SIGN, DEC205 and Langerin receptors, and fused it with PRRSV structural proteins. The results showed that DEC205 targeting is the most successful in improving humoral and cellular responses while not inducing enough protective immunity (29, 30). In a similar approach, the administration of a rAb conjugated to peptides of PRRSV glycoprotein 4 (GP4) was used to target sialoadhesin (CD169), a receptor present in macrophages and monocytes. This study showed the production of anti-GP4 and neutralizing antibodies in immunized and challenged pigs (31). In other strategies, a sdAb specific to the non-structural protein 9 (nsp9) (Nb6) was produced to block viral infection. The authors demonstrated that viral replication was inhibited in a stable Marc-145 cell line expressing Nb6, proving the potential of sdAb (Nb6) as a new form of protection against PRRSV (32). Similarly, a chimeric mouse x pig antibody anti-linear GP5 epitope had neutralizing activity similar to that of the native mAb (ISU25C1) (33).

Certainly, most rAbs reported today are used to seek control of PRRSV. However, there are also other rAbs that can be used to control other diseases. A scFv-Fc (mouse x pig) specific for
| Application | Ab format | Expression system | Species | Main result | Reference |
|-------------|------------|-------------------|---------|-------------|-----------|
| Diagnosis   | sdAb       | E. coli           | Pig     | High specificity and affinity for the capsid protein of PVC type II. | (11)      |
|             | sdAb       | E. coli           | Pig     | High sensitivity and specificity recognizing the capsid protein of POC type II by ELISA. | (12)      |
|             | Porcinated full Ab | HEK293  | Pig     | Recognizes the E2 protein of CSFV. | (13)      |
|             | scFv       | E. coli           | Pig     | Recognition of B. hyodysenteriae for the diagnosis of swine dysentery. | (14)      |
|             | sdAb       | E. coli           | Pig     | Specific recognition of T. solium antigens for the control of cysticercosis. | (15)      |
|             | scFv       | E. coli           | Pig     | Binds to the 3B region of 3ABC non-structural protein of FMDV. | (16, 17)  |
|             | scFv       | Plants            | Cow     | scFv directed at the VP1 protein of FMDV. | (18)      |
|             | scFv       | E. coli           | Cow     | Highly specific to the capsid protein of BIV. | (19)      |
|             | scFv       | HEK293            | Cow     | Detection of HSP65 from M. tuberculosis. | (20)      |
|             | sdAb (VHH) | E. coli           | Cow     | Recognizes B. melitensis strain Rd1 by ELISA. | (21)      |
|             | Full Ab    | CHO               | Cow     | Higher sensitivity to recognize the pathogenic isoform of prion protein (PrPSc) than its scFv form. | (22)      |
|             | scFv       | E. coli           | Chicken | Specific to the NP of AIV. | (23)      |
|             | scFv       | E. coli           | Birds   | Targeted to P phosphoprotein of NDV involved in transcription and replication. | (24)      |
|             | scFv       | E. coli           | Chicken | Differential diagnosis of classical and very virulent strains of IBVD. | (25, 26)  |
|             | scFv       | E. coli           | Chicken | Recognizes oocyst and macrogamont stages of E. tenella for the diagnosis of avian coccidiosis. | (27)      |
|             | Fc-sdAb (Fc-VHH) | E. coli  | Dog     | Recognizes Canine EGFR to label breast cancer cells. | (28)      |
| Prophylaxis  | scFv-Fc    | HEK293            | Pig     | Antigen targeting of important structural peptides for PEDV and PRRSV to dendritic cell receptors. | (29, 30)  |
|             | Full Ab    | HEK293            | Pig     | Antigen targeting of the glycoprotein 4 of PRRSV to CD169 receptor. | (31)      |
|             | sdAb (VHH) | E. coli           | Pig     | Binds to non-structural protein 9 of PRRSV. | (32)      |
|             | Chimeric M x P Full Ab | Sf9 cells | Pig     | Recognizes PRRSV glycoprotein 5 by WB and ELISA, neutralizing activity in vitro. | (33)      |
|             | scFv-Fc    | HEK293            | Pig     | Antigen targeting to Langerin receptor. Enhances humoral and T CD4 responses against PARD. | (34)      |
|             | scFv-Fc    | HEK293            | Pig     | Reduction of RNA from PEDV in feces. | (35)      |
|             | scFv       | E. coli           | Pig     | Neutralizing activity of PEDV in vitro. | (36)      |
|             | scFv       | E. coli           | Pig     | Recognizes the recombinant C subunit of pAPN by ELISA. | (37)      |
|             | Fused sdAb with plg (VHH2) | Yeast  | Pig     | Neutralizing activity in vitro of FMDV and reduced viremia in vivo. | (38)      |
|             | VH3Bs      | Yeast             | Pig     | Neutralizing activity of FMDV in vitro and delayed clinical symptoms and transmission in vivo. | (39)      |
|             | scFv       | Vero              | Pig     | Antigen targeting of ASPV to SLA. | (40)      |
|             | Chimeric M x P Full Ab | Yeast  | Pig     | Growth inhibition of H. parasuis in vitro and partial protection in vivo to prevent Glasser’s disease. | (41)      |
|             | scFv       | E. coli           | Cow     | Blocks cell adhesion of ETEC. Reduced ETEC infection in vivo. | (42)      |
|             | scFv       | E. coli           | Cow     | Inhibits cell adhesion of ETEC by blocking K99 factor, evaluated in vitro. | (43)      |
|             | scFv       | Plants            | Cow     | Diminished ETEC binding ability in calf enterocytes and in horse blood red cells. | (44)      |
|             | scFv       | E. coli           | Chicken | Neutralizing activity against the IBV. | (45)      |

(Continued)
the Langerin receptor expressed on DCs was fused with the spike protein of PEDV and used to immunize pigs. This strategy induced IgG and IgA responses, as well as a CD4 T cell immune response (34). When this rAb was tested in sows, an IgG response but not an IgA response was produced. Piglets born from these sows were challenged, and maternal immunity reduced fecal viral shedding, while clinical signs were unaffected (35). In other reports, a scFv anti-PEDV was tested in vivo and showed neutralizing activity, a reduced cytopathic effect and a reduction in viral replication titers (36). Similarly, a scFv against porcine aminopeptidase N (pAPN) could block the interaction of PEDV with pAPN, the receptor present in the intestinal epithelium, thus inhibiting virus entry to cells (37).

FMDV has also been a focus of rAb production. Harmsen et al. (71) evaluated a sdAb (VHH) as a tool to control FMD in pigs (71). Subsequently, different forms of sdAbs (VHH2s and VHH3s) were produced to increase its half-life and neutralizing activity compared with those of VHH in vitro. In an in vivo experiment, the authors compared VHH2s and VHH3s forms, showing the advantages of VHH3 forms; VHH3s delayed the development of clinical symptoms and FMDV transmission, basically for the doses, route of administration and higher neutralizing activity (38, 39). In an effort to control ASFV, a DNA vaccine encoding a scFv fused with p54 and p30 antigens resulted in less accumulation of fluid in the intestinal loops, inhibiting virus entry to cells.

In contrast to those concerning pigs, there are fewer reports of rAbs being used to improve bovine diseases. Several attempts have been made to block *Escherichia coli* enterotoxigenic (ETEC) cell-adhesion capacity by targeting K99 fimbriae, a colonization factor. An in vivo evaluation of a scFv anti-F5 fimbriae (K99) resulted in less accumulation of fluid in the intestinal loops, inhibiting virus entry to cells.

**Cows**

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indicative of a reduced ETEC infection in neonatal calves (42). Moreover, similar scFvs directed at K99 factor have been evaluated in vitro using horse red blood cells and calf enterocytes (43, 44) where hemagglutination and binding activity in which has been reduced.

Others
A scFv anti-infectious bronchitis virus (IBV) showed neutralizing activity (45); similarly, a scFv-Fc and scFv anti-infectious bursal disease virus (IBDV) reduced viral titers in vivo and in ovo (46, 47). NDV is a highly contagious viral infection that affects poultry and domestic birds. A scFv that recognizes NDV was produced and evaluated in vitro; the Ab showed neutralizing activity that resulted in reduced viral titers, as well as a lower cytopathic effect, in BHK21-infected cells (48). A scFv anti-Eimeria tenella and -E. acervulina produced in pea plants and Nicotiana benthamiana, respectively (49, 50), reduced the number of oocysts in the feces of chickens fed with transformed plants, especially when feeding with pea plants (51). Additionally, IgA rAb anti-E. acervulina antigens have been produced in N. benthamiana plants as potential immunotherapy agents for young broilers in which vaccination is not always successful (50).

In sheep, a DNA vaccine encoding a scFv fused with Rift Valley Fever Virus Gn peptide was directed at DEC205 and CD11c receptors. Compared with targeting to CD11c and untargeted treatment, targeting to DEC205 promoted IFNγ production but showed an inefficient humoral response (52).

RECOMBINANT ANTIBODIES FOR THERAPEUTIC PURPOSES

The use of rAb for therapy has been mainly focused on dogs where personalized treatments are less challenging than those for farm animals. Examples include therapies for cancer and inflammatory processes.

Pigs
An anti-influenza virus antibody was obtained from a human donor after infection with H1N1 swine-origin influenza virus (SOIV). From this donor, a rAb named F16 was expressed and evaluated in several animal models. In mice and ferrets, F16 showed a therapeutic effect after a lethal challenge with H1N1 and H5N1 viruses, respectively (53). Nonetheless, when evaluated in pigs, F16 did not alter viral titers, although it reduced gross lung pathology when challenged (54).

Cows
Bovine mastitis is caused by a variety of pathogens including Staphylococcus aureus. Tissue adhesion involves multiple proteins, including fibronectin-binding factor A and clumping factor A. These two proteins were targeted using a scFv to obstruct the adhesion mechanisms; therefore, they have potential to be used to prevent and treat bovine mastitis (55). Similarly, a sdAb that could recognize and neutralize β-hemolysin from S. aureus was generated and evaluated in vitro to confirm its neutralizing activity (56).

Others
Therapy in poultry is, in most cases, not economically practical. However, the development of rAbs can simplify this practice. In this manner, a full-IgY rAb that can neutralize IBDV provides an 80% protection rate, in contrast to yolk antibody that offers only 40% protection in chickens challenged with IBDV (57). Similarly, a recombinant adenovirus (Ad5) containing the encoding sequence of a neutralizing sdAb specific for the HA1 domain of H5 virus was created. In vivo administration showed a 90–100% survival rate in lethally challenged mice (58). Additionally, a Fab that recognizes distinct HA0 hemagglutinin epitopes showed neutralizing activity and has great therapeutic potential (59). In this manner, a scFv anti-HA fused with truncated protamine (scFv-tP) designed to deliver siRNA was produced (scFv-tP) and evaluated in vitro, showing a reduction in viral titers (60).

In some cases, human medicine can be adapted for use in veterinary medicine. An example is a chimeric dog x mouse rAb derived from a mouse anti-human nerve growth factor (NGF) mAb (72) used to treat chronic inflammation. In vivo, a single dose of this rAb showed effectiveness in reducing chronic pain similar to that of 7 daily doses of meloxicam in dogs, proving it to be a good alternative for prolonged therapeutic treatments (61). A full caninized rAb anti-canine IL-31, lokivetmab, has been produced and evaluated in clinical trials with outstanding results concerning the control of atopic dermatitis (AD) in dogs (62). Compared to a daily dose of 5 mg/kg of ciclosporin, a drug typically used to treat AD, a single dose of 1 mg/kg ameliorated the symptoms of AD for a month (63). In the field of cancer, a scFv anti-canine CD20, a cell surface molecule expressed in normal and tumoral cells, was produced (64). Similarly, a caninized full Ab against epidermal growth factor receptor (EGFR) induces significant inhibition in proliferation in vitro and viability reduction in canine tumor cells that overexpress EGFR (65). Another anti-tumoral strategy is DC-based vaccination; a scFv capable of recognizing canine DCs has been produced for future use in vaccination and therapy (66).

CONCLUSION

The continuous search for alternative ways to control pathogens influenced the application of rAbs in veterinary medicine, which appears to be influenced by the type of animal production. Most reports describing the use of rAbs for diagnosis are concentrated in cows. Their use for treatment is concentrated in dogs, and use for immunoprophylaxis is concentrated in pigs. Unsurprisingly, in the case of pigs, most reports are concentrated on a solution for PRRSV; alternatively, therapeutic rAb is used to identify treatments for dog diseases (Table 1). There is a wide diversity of rAb forms (Figure 1), and not all have been described in veterinary medicine. However, scFv, chimeric and sdAb are the most common forms of rAbs reported to date. In the upcoming years, the use of rAbs for the control of animal diseases will be a reality and will become a significant part of the economic world of pharmaceuticals. In summary, for the first time, the present mini-review describes the progress of rAb use in the
field of veterinary medicine. This technology has not been fully exploited for diseases of economically impactful animals. The development of rAbs has been proven to be a promising tool in the improvement of animal health.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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