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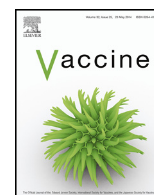

Review

Benefits of using heterologous polyclonal antibodies and potential applications to new and undertreated infectious pathogens

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ABSTRACT

Background: Passive immunotherapy using polyclonal antibodies (immunoglobulins) has been used for over a century in the treatment and post-exposure prophylaxis of various infections and toxins. Heterologous polyclonal antibodies are obtained from animals hyperimmunised with a pathogen or toxin.
Aims: The aims of this review are to examine the history of animal polyclonal antibody therapy use, their development into safe and effective products and the potential application to humans for emerging and neglected infectious diseases.
Methods: A literature search of OVID Medline and OVID Embase databases was undertaken to identify articles on the safety, efficacy and ongoing development of polyclonal antibodies. The search contained database-specific MeSH and EMTREE terms in combination with pertinent text-words: polyclonal antibodies and rare/neglected diseases, antivenins, immunoglobulins, serum sickness, anaphylaxis, drug safety, post marketing surveillance, rabies, human influenza, Dengue, West Nile, Nipah, Hendra, Marburg, MERS, Hemorrhagic Fever Virus, and Crimean-Congo. No language limits were applied. The final search was completed on 20.06.2015. Of 1960 articles, title searches excluded many irrelevant articles, yielding 303 articles read in full. Of these, 179 are referenced in this study.
Results: Serum therapy was first used in the 1890s against diphtheria. Early preparation techniques yielded products contaminated with reactogenic animal proteins. The introduction of enzymatic digestion, and purification techniques substantially improved their safety profile. The removal of the Fc fragment of antibodies further reduces hypersensitivity reactions. Clinical studies have demonstrated the efficacy of polyclonal antibodies against various infections, toxins and venoms. Products are being developed against infections for which prophylactic and therapeutic options are currently limited, such as avian influenza, Ebola and other zoonotic viruses.
Conclusions: Polyclonal antibodies have been successfully applied to rabies, envenomation and intoxication. Polyclonal production provides an exciting opportunity to revolutionise the prognosis of both longstanding neglected tropical diseases as well as emerging infectious threats to humans.
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1. Introduction and methods

The aims of this review are to examine the history of animal polyclonal antibody therapy use, its development into a safe and effective product and its potential future application to humans for emerging viruses and neglected tropical diseases.

A literature search was undertaken by an experienced medical librarian to identify articles on both the safety of polyclonal antibodies and the use for the treatment of rare or neglected diseases. The databases searched included OVID Medline and OVID Embase. The searches contained database-specific MeSH and EMTREE terms in combination with pertinent text-words: polyclonal antibodies and rare/neglected diseases, antivenins, immunoglobulins, serum sickness, anaphylaxis, drug safety, post marketing surveillance, rabies, human influenza, Dengue, West Nile, Nipah, Hendra, Marburg, MERS, Hemorrhagic Fever Virus, and Crimean-Congo. To minimise bias, no language limits were applied. The final search was completed on 20.06.15. These searches yielded 1960 articles. Title searches excluded many irrelevant articles, yielding 303 that were then read in full. Of these, 179 are referenced in this study.

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In 1890, von Behring and Kitasato discovered that sera from rabbits immunised against diphtheria or tetanus were able to protect exposed mice [1]. By 1894, animal derived anti-diphtheria serum was used in humans during a European epidemic [2]; concurrently, Phisalex and Bertrand demonstrated that the blood of horses immunised with *Viper aspis* (the European viper) had antivenin properties [3]. Sera from both humans and animals were subsequently used for management of illness caused by viruses such as measles, varicella and the pandemic Spanish influenza of 1918 [4,5]. Anti-rabies polyclonal antibody preparations from hyper-immunised horses were developed in the early 20th century [6]. In this pre-antibiotic era, serum therapy was also applied to bacterial infections such as pneumococcal pneumonia, meningococcal meningitis and streptococcal scarlet fever [7,8].

The early, crude preparations of serum were often contaminated with animal proteins; as a result, serum sickness and anaphylaxis limited their safe application. Antibiotics against bacterial infections largely superseded serum therapy in the late 1930s and 1940s [7]. During the 1960s, however, there were improvements in enzymatic digestion and purification of equine immunoglobulins, enabling safer polyclonal antibody therapies to be developed for envenomation, rabies exposure and viral infections such as hepatitis A and B, varicella–zoster virus and respiratory syncytial virus (RSV) [8].

The development of monoclonal antibodies (mAbs) in the 1970s enabled large quantities of specific antibody to be produced [8]. The humanisation of mAbs allowed their use in the treatment of chronic disease by repeated administration. However, polyclonal antibodies still proved more effective than monoclonal antibodies in the treatment of many infectious diseases. To date, most monoclonal antibody therapies are produced for either autoimmune conditions (such as systemic lupus erythematosus or Crohns disease) or neoplastic conditions. The only licenced monoclonal antibody to an infectious target is palivizumab, for RSV [9]. In contrast to monoclonal antibodies, polyclonal therapy, by targeting multiple epitopes, can protect against epitope mutation [4]. Additionally, more may be needed of a low avidity monoclonal antibody preparation, compared with a polyclonal preparation, to neutralise a given amount of toxin or pathogen, as is generally the case for antitoxins to snake envenomation [10].

Animal derived polyclonal antibody therapy has been successfully and safely applied to (i) medication overdoses such as colchicine and digoxin, (ii) poisoning by snake, arachnid, marine and plant toxins, and (iii) post-exposure prophylaxis against the rabies virus [11–15]. Polyclonal antibody products can be made in large quantities and cost-effectively to respond to the great endemic demands in Asia and Africa as well as potential pandemic situations, globally.

2. Modern processing dramatically reduces adverse reactions

Adverse reactions in humans to animal-derived polyclonal antibodies are usually due to the presence of highly immunogenic animal proteins. Type 1 hypersensitivity reactions, including anaphylaxis, begin within minutes. Type 3 hypersensitivity, or serum-sickness, results from deposition of immune complexes on small vessels of the skin, joints and kidneys; this can develop at any point over the three weeks it takes to clear the injected antibodies [3]. Antibodies consist of the antigen binding (Fab) fractions, linked by disulphide bonds as $F(ab')_2$, and the crystallisable fraction (Fc). The reactogenic Fc fragment can induce complement-mediated urticaria, angioedema, lymphadenopathy, arthralgia, and nephropathy [3]. Fc removal with retention of the immunogenic $F(ab)/F(ab')_2$ components was first applied to digoxin antiserum in 1976 [16]. The $F(ab)/F(ab')_2$ fragments were isolated initially through salt precipitation and subsequently, since the mid-1990s, via chromatographic purification [17]. Complement mediated anyphylactoid reactions, fever and hypersensitivity have been reduced by careful elimination from the final product of bacterial endotoxins (pyrogens) as well as protein and cell aggregates, through pasteurisation, ultrafiltration and additional chromatography [18]. With this combination of purity and safety advancements, serious adverse events are now rare, even very rare. With equine rabies immunoglobulin (ERIG), anaphylactic shock is reported in less than 1/45, 000 treatments, serum sickness in <0.5% and all-grade adverse events in <5% [3,15].

Ammonium sulphate precipitation of the $F(ab')_2$ was the main method of purification until the mid-1990s when chromatography was introduced [19]. Examples of current production techniques are for that of the rabies equine immunoglobulin produced by QMSI (Queen Saovabha Memorial Institute, Bangkok) and Favirab (Sanofi-Pasteur) as well as equine snake antivenins [19,20]. Briefly, the techniques involve hyper-immunisation of the source animal daily for several days and collection of sera 2–4 weeks after the last injection, which enables antibody affinity maturation, resulting in a highly avid, concentrated product, which reduces the co-administered load of contaminant animal proteins [21].

The purification process often starts with an anion-exchange chromatography step which isolates the immunogenic horse IgG subclass, and removes other immunoglobulins, proteins, cell aggregates and contaminants [19]. Enzymatic cleavage or digestion by low pH pepsin of IgG to Fc and active $F(ab')$ or $F(ab')_2$ regions reduces adverse reactions [17]. A second anion-exchange chromatography excludes the Fc fragments, protein and cell aggregates which result from low pH pepsin digestion, as well as bacterial pyrogens [22]. European guidelines require animal immunosera that are test-negative for pyrogens [23]. A final pasteurisation step involves heat-treatment for 10 h at 60 °C, which destroys viruses, and thermocoagulates excess proteins [20]. High performance liquid chromatography is used to control purity of the final product; usually 90% of the content is covalent $F(ab')_2$, 5% F(ab) fragments, and <0.5% are polymers/aggregates [18]. Importantly, immunoreactivity remains intact after these steps [19]. The final antiviral antibody titre is determined for example by ELISA or seroneutralisation assays for antiviral immunoglobulins. Variations in the production process include application of potassium sulphate for $F(ab')_2$-Fc separation, and precipitation of non-immunoglobulin proteins, caprylic acid precipitation to purify whole IgG (resulting in near total exclusion of albumin, less activation of complement as well as lower total protein/protein aggregates compared to ammonium sulphate precipitation), and centrifugation to eliminate cellular elements and proteins [3,24–27]. Preservatives are added to the final products to prevent bacterial and fungal contamination [28]. Refrigeration and avoidance of prolonged storage avoids protein/cell reaggregation and precipitation [28].

Apart from pasteurisation, steps that neutralise pathogens include low-pH pepsin hydrolysis and high-temperature caprylic acid precipitation, both of which lipolyse enveloped viruses such as Herpes, Sindbis and West Nile viruses; and ultra/nano filtration e.g. using 0.22 μm gauge filters, for virus and bacterial removal [4,29,30]. Subculturing can confirm bacterial sterility [4]. The WHO, in recognition of some inconsistency in antivenin production quality, has released the 'WHO Guidelines for the Production, Control and Regulation of Snake Antivenom' [31].

Large relative molecular mass (Mr) bivalent antibodies (IgG and $F(ab')_2$ fragments) have a smaller volume of distribution and a longer half-life in the human body than the lower Mr $F(ab')$ fragments [32]. Both IgG and $F(ab')_2$ fragment elimination occurs mainly by formation and elimination of immune complexes (catabolism), whereas $F(ab')$ is cleared renally [33].

The longer half-life means that antivenins made from IgG and $F(ab')_2$ persist, which reduces rebound symptoms of envenomation associated with F(ab′) antitoxins [34]. Additionally, the preservation of large Mr antivenins in the vascular compartment draws venin out of tissues into the bloodstream to form antivenin-venom complexes, promoting venom clearance [35]. However, recent human pharmacokinetic data suggests that $F(ab')_2$ also extravasates into tissues [36,37]. This tissue penetration may assist with neutralizing viruses infecting various organs.

Cleaving of the Fc fragment, whilst reducing adverse events, possibly reduces it's potentiating effect on generation of natural immunity towards a pathogen or toxin. Fc interaction with antigen-presenting may stimulate and promote development of active immunity towards the exposure [38,39].

These features may have implications for the selection of molecule type in the production of polyclonal immunotherapy, particularly for post-exposure prophylaxis against viruses such as Mers-CoV and Ebola. Dosing intervals are longer and therefore dosing regimens more pragmatic with IgG and $F(ab')_2$ and IgG also may promote development of host immunity. However, this needs to be balanced against the increased risk of hypersensitivity reactions from the Fc molecule.

Half-time of elimination (t1/2) of the product in the plasma compartment was analyzed after intravenous (IV) administration of $F(ab')_2$ against avian influenza A H5N1 in 3 healthy volunteers receiving 1 dose and in 10 healthy volunteers receiving 5 doses [40]. The plasmatic elimination of $F(ab')_2$ after one IV infusion had a mean t1/2 of 16.77 h and after 5 infusions 24 h apart, a mean of 10.89 h. These results indicated the persistence of equine $F(ab')_2$ in the plasma for the duration of the therapeutic protocol with evidence of a slight accumulation between day 1 and day 5. After the fifth infusion, equine $F(ab')_2$ remained detectable by ELISA in plasma, (above > 1 μg/mL) for between 3 to 14 days. These results were consistent with another human study of intravenous equine $F(ab')_2$, with a plasma t1/2 of 14.2 h and a later catabolism of $F(ab')_2$ with a t1/2 of around 7 days[37]. Thus, the protection induced may continue several days after the end of the treatment protocol, up until the patient's immunity generates host antibodies.

3. Management of rare secondary effects

A meta-analysis of seven studies and a Cochrane review each concluded that adrenaline premedication, but not other agents, significantly reduced early adverse reactions [41]. Additionally, early use of adrenaline for anaphylactic reactions post antivenin is effective [42].

Ovine (sheep-derived) products may be somewhat less reactogenic than equine products, but the latter are more economical to produce given the larger amounts of sera available, and have a longer half-life, reducing requirement of re-administration [43,44]. Equine antivenin may also have superior antitoxin effects compared to ovine[45].

There are no recorded cases of viral or prion transmission from equine-derived antitoxins and current processes aim to preserve this safety record [29]. Both initial measures (e.g. donor selection, epidemiological exclusion, quarantine, health status of the animal) and processing of final product reduce the risk of a contaminating virus. Animals are contained within closed flocks or maintained in areas free of insect vectors of certain arboviruses. For example, rattlesnake, viper and digoxin antitoxins are manufactured in South Australia, a region free of prions and many viral pathogens [46]. Source animals may also be vaccinated against local pathogens such as rabies, anthrax and viral equine encephalitides [31]. Molecular diagnostic screening of animals for viruses may be performed [29,31]. Record-keeping and regular stock inspections contribute to quality control [4]. The rigor of the application of such processes varies by resource availability e.g. in resource-challenged countries where much of the world's antivenin/antitoxin is both required and produced, not all safety measures may be employed [15,47]. Polyvalent (source animal immunised with more than one venin) polyclonal antivenins have higher rates of adverse reactions than monovalent (source animal immunised to just one venin) polyclonal antivenins, e.g. 24% vs. 9% for Australian produced CSL snake antivenins, due in part to the larger volumes required to be administered for treatment with the polyvalent rather than monovalent polyclonal antibodies [48].

4. Efficacy and safety of modern polyclonal immunoglobulin products

Clinical studies have demonstrated the safety and efficacy of equine immunoglobulin. These therapies can even be given to pregnant women as no passage of $F(ab')_2$ across the placenta is expected and thus no teratogenicity anticipated[49].

Of 7660 Filipino recipients of $F(ab')_2$ equine rabies immunoglobulin (ERIG), (Favirab, Sanofi Pasteur, Lyon, France) only two developed rabies; neither had received post-exposure prophylaxis (PEP) strictly as per the WHO guidelines [50,51]. Of the151 subjects in this cohort who sustained bites from laboratory-confirmed rabid animals, there were no reported cases of rabies. Of 193 persons bitten by rabid dogs in the Philippines, there was just a single recorded PEP failure; due to location of the bite (on the lip) local ERIG infiltration was difficult, and, against official protocol, she received a mix of intradermal and intramuscular rabies vaccine [52]. These results emphasise the importance of adherence to WHO rabies guidelines in administering PEP.

The safety of current ERIG products has also been demonstrated in clinical trials and post marketing surveillance. Of over 12,000 ERIG recipients in the Philippines, Thailand and India, 0.3 and 1% of recipients developed local reactions and 0.03–3% had systemic reactions [15,52–54]. Some of these reactions may have been due to co-administered tetanus toxoid.

A retrospective review of over 70,000 patients in Thailand who received either human rabies immunoglobulin (HRIG) (59.6%) or ERIG (40.4%) demonstrated that 1.83% of ERIG recipients had an adverse events, versus 0.09% of HRIG recipients; however the broad date ranges included ERIG produced both before and after the introduction of modern purification techniques [55]. Serum sickness was reported in 0.72% of ERIG recipients vs. 0.007% of HRIG recipients, and no deaths were reported.

Antivenins are also effective. While there are few randomised prospective controlled human studies, mice studies, retrospective human studies and human case reports provide evidence for effective and safe treatment of life-threatening envenomations and coagulopathy [56,57].

Several human reports confirm efficacy of various snake antivenins (Tables 1 and 2) [33,58–62].

Efficacy of rattlesnake antivenin has been studied, particularly in the USA, where rattlesnakes predominate; introduction of rattlesnake whole IgG polyvalent crotalid antivenin (ACP) reduced mortality from up to 25% to <0.5% when delivered in a health care facility in the United States[63]. A 10-year retrospective chart review revealed the fractionated Crotalidae polyvalent immune F(ab′) (CroFab®) to be more effective at avoiding fasciotomies than the whole IgG product ACP[64]. A 12 year review of CroFab® revealed a response rate of 77% amongst 24 cases of severe envenomation, including neurotoxicity, whilst in another series of 28 severe envenomations, all responded to CroFab® [65,66]. A literature review of controlled and observational studies confirmed the

Table 1
Efficacy of various snake antivenins.

Study	Efficacy	Mild reactions (%)	Serum sickness	Anaphylaxis
Otero et al. *Bothrops* whole IgG [48]	67/67 treated: coagulopathy reversed 48H	15–24	nil	nil
Thomas et al. *Bothrops*F(ab′) [49]	45 cases treated vs. 27 no treatment Treatment favoured: • mortality (nil vs. 22%) • neurological complications (nil vs. 3%) • shorter hospitalisation • fasciotomy requirement unchanged		4.4%	2.2%
Thomas et al. *Bothrops* F(ab′) [50]	68 treated vs. 64 untreated Treatment favoured: • Mortality (nil vs. 12.5%) • Reduced coagulopathy (nil vs. 17.5%), • Reduced thrombosis (nil vs. 28.1%) • Shorter hospital stay (5 days vs. 12	5.9	1.5%	1.5%
Ha et al. *Bungarusmulticinctus* F(ab′) [51]	27 treated vs. 54 untreated Treatment favoured (in days): • Duration of limb paralysis (2.2 vs. 7.5) • Diaphragm palsy (1.6 vs. 7.0) • Duration of ptosis (3.5 vs. 6.3) • Duration of ventilation (2.3 vs. 8.6) • ICU stay (6.1 vs. 11.6)	7.4	nil	nil
Chippaux et al. Polyvalent F(ab)$_2$IPSER AFRICA (Pasteur Merieux Connaught, Lyon, France) [52]	223 patients Cure rate 96.3% Fatality rate 1.3%	6.3	0.4%	0.4%
Chippaux et al. Polyvalent F(ab)$_2$ FAV-Africa (Pasteur Merieux Connaught, Lyon, France)[53]	46 patients All survived	4	nil	nil
Chippaux et al. Polyvalent F(ab′)$_2$ AfricanAntivipmyn (Laboratorios Silanes, Mexico City, Mexico) [54]	289 patients Survival rate 99.3% Fatality rate 0.7%	19	nil	nil

Table 2
Safety estimates from meta-analysis of non-crotalidae antivenin observational and controlled trials.

	95% CI Mild Reactions A vs. B	95% CI Serum Sickness A vs. B	95% CI Anaphylaxis A vs. B
Pre-1995 all antivenin [A: 111 cases] vs. post 1995 all antivenin [B: 2873 cases]	25.2–33.2%	2.7–4.2%	−1.5–2.5% (no significant difference)
Whole IgG [A: 899 cases] vs.F(ab′)/F(ab′)2 [B: 2085 cases]	12.8–16.2%	1.3–2.0%	7.2–8.6%
Post 1995: Whole IgG [A: 874] vs. F(ab′)/F(ab′)2 [B: 1999]	14.9–18.0%	1.9–2.5%	7.7–9.2%

efficacy and safety of CroFab® [67]. Crofab® has also been demonstrated to be effective in paediatric patients [68,69]. However, a South American study demonstrated a locally produced whole IgG antivenin was more effective than two F(ab′)$_2$ preparations but with more anaphylactoid reactions [70]. Amongst the fractionated products, a comparison of F(ab′) and F(ab′)$_2$ rattlesnake antivenin demonstrated a significant reduction in late coagulopathy in those who received F(ab′)$_2$ compared to F(ab′) (29.7% vs. 10.3%) due to the longer half-life of the divalent over the monovalent product [34]. This advantage of F(ab′)$_2$ over F(ab′) has been demonstrated for other antivenins [71,72].

Epidemiological data from Europe demonstrates that the introduction of antivenin has resulted in a several-fold decrease in snakebite mortality [73]. Australian snake antivenins have been considered more effective at neutralising the neurotoxic effects than the pro-coagulant effects, and plasma transfusions are recommended along with antivenin [46,74,75]. This limitation of current Australian antivenins has been disputed, however [76].

Safety of snake antivenins has improved with antibody fractionation and modern processing. Whole IgG rattlesnake antivenin (e.g. Antivenin Crotalidae Polyvalent ACP) was associated with a 18–50% frequency of immediate and delayed reactions [77–83]. This was more than halved after the removal of Fc and implantation of modern purification methods (e.g. Crofab®) [63,67,84–93,65,94].

A meta-analysis of CroFab® revealed an immediate hypersensitivity rate of 8% and serum sickness of 13%[95], with even lower rates reported in a subsequent study of 340 cases: <2% immediate hypersensitivity reactions and 5% serum sickness [96]. Likewise, the adverse event rate of bothrops antivenin improved from 25 to 82% for ammonium sulphate precipitated whole IgG preparations to 11–28% for caprylic acid precipitated whole IgG and 12–36% for F(ab′)$_2$ antivenin [26,28].

A meta-analysis was conducted by us, the authors, of 30 observational and controlled studies of various snake antivenins, excluding rattlesnake antivenin for which a meta-analysis has been reported above [27,33,43,48,59,60,62,70–72,97–116] (Table 2). A simple linear weighting by the sample size of each study was applied. The weighted mean and standard deviation for each set of studies were compared using a formula to isolate the effect of the independent variable (the anti-venin) given to an otherwise similar population distribution. We determined an estimate of the percentage difference with $z = 1.96$ to give 95% confidence intervals, which are reported below. This analysis demonstrates that F(ab′) and F(ab′)$_2$ are safer than whole IgG and that there was a reduction in adverse events after introduction of purification techniques in the mid-1990s, with the exception of no reduction in anaphylaxis pre and post 1995 for all types of antivenins, combined.

There are approximately 1.5 million scorpion envenomations annually resulting in about 2600 deaths, from autonomic overstimulation and/or an overwhelming inflammatory response. A randomised controlled trial (RCT) of anti-scorpion $F(ab')_2$ in Arizona revealed a significant difference in the following: rapid symptom resolution, midazolam requirement and plasma venom levels, compared to placebo [117]. A meta-analysis of 4 RCTs and 5 observational studies demonstrated effectiveness in new world (American) scorpion envenomations but not in the old world[118]. Adverse events were higher in non-randomised trials, e.g. serum sickness occurred in 57% of those treated in one study, but lasted only 3 days, and antivenin was highly effective, reducing symptom duration from 22 h to 31 min [119]. Adverse events were infrequent in the RCTs (0–2%). However, this meta-analysis included a 2011 Indian ('old world') controlled trial where scorpion $F(ab')_2$ antivenin reversed clinical envenomation more effectively than no antivenin [120]. Three other old-world studies not included in the meta-analysis have demonstrated efficacy. An uncontrolled Indian study of scorpion $F(ab')_2$ antivenin where 41/48 (85.4%) responded, with one mild reaction[121]; a prospective case-control series from India of 62 patients under the age of 18 years which demonstrated that dopamine and dobutamine requirement was reduced and recovery was faster in those who received scorpion antivenin [122] and a controlled trial from Saudi Arabia that reported a reduction in mortality from 4–8% to <0.05%, with mild reactions only (13.9%) [123]. In a review of 10 studies, only this Indian uncontrolled trial of scorpion $F(ab')_2$ antivenin revealed efficacy, as opposed to whole IgG in all the other trials [124]. However, withdrawal of IgG scorpion antisera in the USA in 2002 with no alternative substituted, resulted in a 5-fold increase in ICU admissions for stings [125]. Saudi Arabian data demonstrated a reduction in deaths from 1.7% to nil, in pulmonary oedema from 11.1% to 1.2% and in cardiac arrest from 7.4% to 0.4% after introduction of scorpion antivenin in 1991 [126].

Spider antivenin is also effective and safe. A review of whole IgG funnel-web spider antivenin use in Australia reported a complete response in 97% of 75 recipients, with only one local reaction, one case of anaphylaxis and one case of serum sickness [127]. Whole IgG black widow spider (lactodectrus) antivenin reduced durations of symptom and hospitalisation in moderate to severe envenomation in a US review [128,129]. Intravenous (as compared to intramuscular) administration was associated with high rates of anaphylaxis (e.g. 9% in 1989) and serum sickness (33%) prior to reducing the speed of administration, which reduced the total adverse event rate to less than 3% [130]. Equine derived $F(ab')_2$ black widow antivenins have been developed and are being tested [131]. An Australian study of 95 cases of redback spider antivenin $F(ab')_2$ yielded 4 cases of anaphylaxis (4.2%) and a serum sickness rate of 9.3% [132]. Overall, polyclonal antibodies used as antidotes are also reportedly safe. A study of 717 patients who received anti-digoxin F(ab′) revealed an allergy rate of 0.8% [133].

5. Polyclonal antibodies for emerging and neglected viral diseases

Polyclonal serum therapy is emerging as potentially applicable to a range of viruses for which there are limited therapeutic options.

Highly pathogenic avian influenza (HPAI) viruses such as H5N1 and H7N9 are new targets for polyclonal $F(ab')_2$ immunoglobulin therapy. Stockpiles of effective product for prophylaxis and treatment are required in anticipation of epidemics. The neuraminidase inhibitor oseltamivir remains the mainstay of treatment with an overall reduction of mortality risk reported of 49% [134]. Reports of oseltamivir resistance in HPAI H5N1 as well as in seasonal human influenza strains suggests complementary treatment options are necessary [135,136].

The World Health Organisation (WHO) recognises the potential role of serum therapy for influenza pandemic planning, given its historical success in infectious outbreaks [137]. Use of convalescent plasma during the 1918 Spanish influenza epidemic of 1918 apparently reduced mortality by 50% [5,138]. Convalescent plasma has been used in two cases of HPAI H5N1, both of whom survived [139,140]. Since the first human case of HPAI H5N1 in 1997, the WHO has recorded 718 human infections with 413 deaths as of 26 January 2015, a case fatality rate of 57.5% [141]. All cases have been in Africa, Asia or the Middle East, or travellers returning [142]. Survivors produce demonstrable neutralising antibodies [143]. Whilst there is currently only limited person-to-person transmission, antigenic shift (via reassortment with circulating human viruses) could confer this, setting the stage for a devastating pandemic [144].

In vivo proof-of-concept studies of equine polyclonal $F(ab')_2$ to HPAI H5N1 have been conducted in mice [145]. The product was able to prevent infection after an intranasal HPAI H5N1 challenge. Sero-neutralising assays and haemagglutination inhibition tests confirmed the potent neutralising abilities of equine polyclonal anti-HPAI H5N1 $F(ab')_2$ preparations in mice[145]. In another study, four H5N1 avian influenza equine $F(ab')_2$ preparations demonstrated cytopathic effect against cultured Madin–Darby canine kidney (MDCK) cells infected with H5N1 and protected mice against lethal challenge, both given prior (prophylaxis) or post (therapeutic) exposure [146]. These studies concur with earlier mice studies of polyclonal antibodies to seasonal influenza A. In one such study, mice were immunised with the M2 antigen of influenza A, anti-influenza IgG was obtained and intravenously injected into other exposed mice leading to 100% survival with high dose (320 micrograms) IgG [147].

A phase 1 study in 16 healthy young human males aged 21–40 years who received intramuscular polyclonal $F(ab')_2$ to HPAI H5N1 yielded no serious adverse events, no changes in blood or urinary parameters, and only one febrile reaction, likely related to the product; there was also evidence of clinical benefit as assessed by seroneutralising and haemagglutination inhibition testing of the subjects' plasma samples [40]. As ethically no placebo-controlled efficacy studies in humans can be performed, further effectiveness data will have to be gathered from compassionate case-based use.

Other fatal avian influenza viruses are emerging in people; in March 2013 the H7N9 avian influenza virus emerged in China and spread to Hong Kong, Taiwan and Malaysia; 718 cases and 413 deaths have been reported to date [148]. Both HPAI H5N1 and H7N9, have demonstrated rare person-to-person transmission [148–151]. In May 2013, the first case of avian influenza A H6N1 was reported in Taiwan [152]. Three human cases with avian influenza H10N8 were reported in China between December 2013 and February 2014 [153–165]. Both of these are low pathogenic strains currently, but the potential remains for acquisition of mutations that may increase pathogenicity. Several studies have suggested the potential role of respiratory tract administration of polyclonals to prevent and treat seasonal influenza infection, suggesting a potential for these to be applied to avian influenza strains [156–158].

As well as the threat of new avian influenza viruses, the Middle Eastern respiratory syndrome coronavirus (MERS-CoV) that emerged in 2012 has pandemic potential. MERS-CoV causes severe respiratory illness and, sometimes, renal injury [133,159]. Whilst most cases have occurred in several Middle Eastern countries, particularly Saudi Arabia, cases have been reported from Korea, Europe and North Africa [160,161] Of the 1368 reported cases of Mers-CoV to WHO by 7 July 2015, 487 have died (35.6%); but a large proportion of cases may go undiagnosed [162]. There appear to be a mix of zoonotic (bat and/or camel) and human sources for transmission; person-to-person transmission has been particularly documented in health-care settings [162–164]. There is

neither a specific treatment nor a licenced vaccine. In a recent study, serum of Egyptian dromedary camels who were seropositive for Mers-CoV was administered to mice infected with Mers-CoV, with Australian camel sero-negative sera serving as a control [165]. Mers-CoV seropositive camel serum given both pre- and post-exposure protected infected mice from weight loss, diminished lung histological changes and accelerated virus clearance. Polyclonal antibodies could provide post-exposure prophylaxis for close contacts as well as a therapeutic strategy for those severely affected by MERS-CoV. One potential setting for the development of an epidemic of either H5N1 or MERS-CoV is the annual HAJJ pilgrimage in Saudi Arabia, where preparations for a potential MERS outbreak are being refined [166].

Equine polyclonal antibody therapies could also be developed for other widespread and severe neglected tropical diseases e.g. the viral haemorrhagic fevers Crimean-Congo Haemorrhagic Fever, Dengue, Ebola and Marburg; bat-transmitted viruses such as Nipah and Hendra, as well as Lassa virus, West Nile Virus (WNV) and severe acute respiratory syndrome-associated coronavirus (SARS). The WHO has reported 27,705 cases of Ebola with over 11,269 reported deaths in the 2014–2015 West African epidemic [167].1 A stockpile of polyclonal antibodies may form part of epidemic preparation.

Animal studies exist demonstrating efficacy of polyclonal antibody therapy for various neglected tropical viral diseases. Unimmunised hamsters that received WNV-immune hamster antisera one hour before and 24 h after a WNV challenge were protected from lethal WNV infection [168]. Duck egg polyclonal $F(ab')_2$ neutralised Andes virus (the primary agent for pulmonary hantavirus syndrome in South America) in vitro; hamsters given the polyclonal $F(ab')_2$ post-exposure had improved survival compared to controls [169]. Polyclonal antibodies against the Marburg and Ebola filoviruses were acquired from non-human primates (NHPs) that survived filovirus challenge and, when given post-exposure, prevented disease and death in virus-challenged NHPs compared to controls [170]. Similar results were obtained from injecting Ebola infected mice with polyclonal sera from *E. bola* immunised mice [171]. Other studies of mice, monkeys and guinea pigs have demonstrated purified equine whole IgG has prolonged survival against Ebola [172–176]. Equine $F(ab')_2$ has been verified to protect both mice and hamsters from development of infection with SARS-CoV infection when given prophylactically and reduced lung viral titres when given therapeutically, compared to controls [177–179].

6. Conclusions

In summary, clinical studies have demonstrated the efficacy of animal-derived polyclonal antibody therapies against various infections, toxins and venoms. The safety of current products has been demonstrated in clinical trials and post marketing surveillance. When WHO guidelines are followed in administering rabies equine polyclonal antibodies, post-exposure prophylaxis is highly effective in averting rabies. Antivenins are also safe and effective for snake and arachnid bites, with available data suggesting a positive effect against scorpion bites. Antidotes are also reportedly safe and effective. Polyclonal antibodies are being developed against viruses with epidemic and pandemic potential for which prophylactic and therapeutic options are currently limited, such as avian influenza and other zoonotic viruses. Preclinical studies as well as phase 1 safety studies against avian H5N1 influenza, are promising and the technology exists to rapidly apply the methods of polyclonal production against a wide range of pathogenic antigens, providing an exciting opportunity to revolutionise the prognosis of both longstanding neglected tropical diseases as well as emerging viral threats to humans.

Conflict of interest statement

The authors have no conflict to declare.

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