

RESEARCH ARTICLE

Blood Groups in the Species Survival Plan[®], European Endangered Species Program, and Managed *In Situ* Populations of Bonobo (*Pan paniscus*), Common Chimpanzee (*Pan troglodytes*), Gorilla (*Gorilla* ssp.), and Orangutan (*Pongo pygmaeus* ssp.)

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Blood groups of humans and great apes have long been considered similar, although they are not interchangeable between species. In this study, human monoclonal antibody technology was used to assign human ABO blood groups to whole blood samples from great apes housed in North American and European zoos and *in situ* managed populations, as a practical means to assist blood transfusion situations for these species. From a subset of each of the species (bonobo, common chimpanzee, gorilla, and orangutans), DNA sequence analysis was performed to determine blood group genotype. Bonobo and common chimpanzee populations were predominantly group A, which concurred with historic literature and was confirmed by genotyping. In agreement with historic literature, a smaller number of the common chimpanzees sampled were group O, although this O blood group was more often present in wild-origin animals as compared with zoo-born animals. Gorilla blood groups were inconclusive by monoclonal antibody techniques, and genetic studies were inconsistent with any known human blood group. As the genus and, specifically, the Bornean species,

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orangutans were identified with all human blood groups, including O, which had not been reported previously. Following this study, it was concluded that blood groups of bonobo, common chimpanzees, and some orangutans can be reliably assessed by human monoclonal antibody technology. However, this technique was not reliable for gorilla or orangutans other than those with blood group A. Even in those species with reliable blood group detection, blood transfusion preparation must include cross-matching to minimize adverse reactions for the patient. *Zoo Biol* 29:1–18, 2010. © 2010 Wiley-Liss, Inc.

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INTRODUCTION

Blood group determination, or “blood typing,” is performed within a species for management purposes of genetic variation, pedigree validation, forensics, and determination of taxonomic relationships [Socha, 1981; Crouse and Vincek, 1995; Saitou and Yamamoto, 1997; Penedo, 1993]. Medically, this technique is the cornerstone of effective blood transfusion as it categorizes potential donors to reduce likelihood for donor–recipient mistransfusion [Socha, 1981; Socha et al., 1984; Feldman, 1999; Giger, 2000; Seltsam et al., 2003; Stieger et al., 2005]. Equally important, although often misunderstood, is cross-matching. This procedure refines the limited donor pool for a specific patient by assessing for the presence of circulating antibodies in the recipient to red blood cell (RBC) antigens of the donor, including but not limited to ABO and Rh-factor [Feldman, 1999; Knottenbelt, 2002; Stieger et al., 2005]. Even though these clinical procedures are readily available in human medicine, the technology for veterinarians has been focused largely in domestic dogs, cats, and horses, and to a lesser extent ferrets and llama [Penedo, 1993; Giger, 2000; Knottenbelt, 2002; Hohenhouse, 2004; Giger et al., 2005; Stieger et al., 2005]. For animals housed in zoos, it is not only the patients at risk but also the donors, as these animals nearly always require sedation to even collect the blood sample for initial patient screening. Therefore, to date, for animals housed in zoos, transfusion procedures have been reliant on cross-matching [Greenberg et al., 1999].

Blood groups in humans were identified in the early 1900s and subsequently in domestic animals by the 1950s and 1960s, although the latter remained less specifically defined until the 1980s [Eyquem et al., 1962; Feldman, 1999; Giger, 2000; Knottenbelt, 2002]. Blood group investigation was limited in nonhuman primates—except in common chimpanzees until 1940, which follows the discovery of Rh-factor and its impact on human neonatal medicine [Socha et al., 1984; Kendig, 2007]. The great apes were considered potential human models with substantial numbers of studies in laboratory-housed chimpanzees in 1925, and limited number of zoological or *in situ* samples for chimpanzees and other great ape species in the 1960s and 1970s [Weiner and Gordon, 1960; Eyquem et al., 1962; Wiener et al., 1963, 1976; Weiner and Moor-Jankowski, 1972; Socha et al., 1984, 1993; Moor-Jankowski et al., 1975; Socha, 1981]. The great ape literature in this field then became quiescent, until DNA-based genotyping led to molecular description of the ABO blood group genes and interpretive phylogeny in nonhuman primate evolution during the last 20 years [Yamamoto et al., 1990; Yazer, 2005]. In the last decade, these molecular techniques have entered mainstream clinical techniques for even domestic pet species [Feldman,

1999; Giger et al., 2005; Stieger et al., 2005]. In some of these studies, it was revealed that certain blood groups predominate within geographic human populations and domestic animal breeds; so, knowledge of these patterns more quickly provides an appropriate donor selection pool [Weiner and Moor-Jankowski, 1972; Giger, 2000; Knottenbelt, 2002; Hohenhause, 2004; Stieger et al., 2005].

Determination of ABO blood group is based on the carbohydrate-based antigen (H) on the surface of RBCs [Yamamoto et al., 1990; Stieger et al., 2005; Yazer, 2005]. For primates, including humans, blood group studies focused largely on the ABO gene, which causes the conversion of the H antigen to either A- or B-antigen [Crouse and Vincek, 1995; Kermarrec et al., 1999]. In primates, the blood group A is considered the ancestral phenotype [Saitou and Yamamoto, 1997; Kermarrec et al., 1999] and blood group O is currently the most common phenotype in humans [www.bloodtyping.com, accessed February 2006 and March 2010]. Anti-A or Anti-B antibodies are produced against the converted carbohydrate in individuals that themselves do not have the A- or B-antigen on their RBCs. As described by Landsteiner, these antibodies circulate in the serum and destroy donor RBCs presenting with the converted antigen [Socha et al., 1984]. Misreading or malfunction in the ABO gene sequence produces no conversion of H; therefore, no antibodies are produced and group O blood occurs [Yamamoto et al., 1990; Kermarrec et al., 1999; Yazer, 2005]. In humans, antibodies to these carbohydrates are present innately, presumably arising from environmental or dietary exposure to gastrointestinal bacteria or plant epitopes which have structural components that resemble the RBC antigens [Knottenbelt, 2002; Kindt et al., 2007]. Ongoing exposure to these sources is thought to induce individual sensitization and population changes in blood group profiles. Although primarily associated with RBCs, the ABO blood group antigens can be expressed in other cells and secreted through body fluids, such as saliva, which has been confirmed in humans and great apes [Weiner and Gordon, 1960; Wiener et al., 1963; Moor-Jankowski and Wiener, 1964; Socha et al., 1984; Crouse and Vincek, 1995]. Although blood groups are frequently named A, B, or O in great apes and other mammals, it is important to recognize that these antigens are not interchangeable with the human blood group A or B at the molecular level, but rather indicate reactivity to anti-A or anti-B antibodies for blood groups A and B respectively, or neither in blood group O [Eyquem et al., 1962; Socha and Moor-Jankowski, 1978; Socha et al., 1984; Giger, 2000; Knottenbelt, 2002; Hohenhause, 2004; Stieger et al., 2005].

When indicated, whole blood transfusions can be life-saving in any species. This technique is integral to human and veterinary medicine. However, routine blood transfusions in dogs and cats was not common until recently, and current veterinary literature has cited this procedure as handled primitively in all species but these two [Socha et al., 1984; Feldman, 1999; Giger, 2000; Hohenhause, 2004; Stieger et al., 2005]. Clearly, the many exotic animals housed in zoos that could need transfusion have blood groups that remain minimally explored, even in the great apes that have medical care essentially modeled on humans. Poorly executed transfusions can produce immune reactions that result in the destruction of RBCs and compromise already weakened patients, and can result in death of the recipient [Socha et al., 1984; Feldman, 1999; Greenberg et al., 1999; Knottenbelt, 2002; Sheppard and Hillyer, 2007]. Naively, in veterinary medicine, it is often considered that a single transfusion can be performed without blood group determination or

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cross-matching, as generally the initial exposure does not immediately produce circulating antibodies in the recipient [Socha et al., 1984; Feldman, 1999; Giger et al., 2005;]. In species where blood groups have not been defined, reliance on use of the single transfusion is especially invoked, even when use of simple cross-matching techniques could improve selection of a potential donor and minimize future blood mistransfusion. For example, although it may not be the immediate plan to transfuse an individual more than once, the clinical progression of the patient may warrant repeated whole blood infusions or pregnancy may be desired at a future date. Additionally, previously unknown medical history may have hidden prior sensitization episodes, such as trauma; early pregnancy loss or prior multiple fetal gestations; or, in some species, natural antibodies are present without prior exposure [Weiner et al., 1977; Feldman, 1999; Knottenbelt, 2002; Hohenhouse, 2004; Kendig, 2007]. In any of these situations, blood mistransfusion issues could occur with the first clinical blood transfusion for an animal.

Specifically, pregnancy may sensitize the dam or fetus through alloimmunization [Weiner et al., 1977; Kendig, 2007] owing to incompatible blood groups of the dam and fetus. In domestic mammals which are strongly reliant upon colostral transfer of maternal antibodies, the maternal and fetal circulations are separated largely by placental barriers [Franks, 1962]. In these species, pregnancy itself will not be compromised by the production of maternal antibodies toward fetal antigens [Franks, 1962]. However, at colostral nursing, these neonates can be exposed to a marked concentration of hemoactive antibodies from the maternal circulation and rapidly present clinical signs consistent with transfusion of incompatible blood groups [Franks, 1962; Swisher et al., 1962; Stieger et al., 2005]. In primate species, the intimate contact of the maternal–fetal circulation by way of the hemochorial placenta can permit *in utero* fetal exposure to these blood antibodies from a sensitized dam [Franks, 1962]. As occurs in human neonatal isoerythrolysis [Weiner and Gordon, 1960; Socha et al., 1984; Kendig, 2007], fetal death or birth of weakened neonates with infant mortality has been documented in common chimpanzees [Weiner et al., 1977] and orangutans [Socha and van Foreest, 1981]. Prevention or prophylactic addressing of this problem relies upon accurate knowledge of blood groups and circulating antibodies, most notably ABO and Rh-factor, from both parents [Kendig, 2007].

In zoological mammalian species, and especially nonhuman primates, each transfusion episode should be handled consistently and include blood group determination whenever possible, and in all cases, major and minor cross-matching [Feldman, 1999; Knottenbelt, 2002]. As blood groups are not well defined in zoo species, reliance on cross-matching to detect circulating hemoactive antibodies has been the norm, although blood groups cannot be determined by this technique [Knottenbelt, 2002]. In positive major cross-matching, effects of the recipient serum against the donor RBCs are considered potentially more damaging than a positive minor cross-matching [Knottenbelt, 2002]. Because zoo species do not have dedicated donors and especially not ones that can be excluded from the breeding population and potentially sensitizing pregnancies, it is important to also evaluate donors at each transfusion regardless of if they have previously successful donation history [Feldman, 1999].

In progressive domestic veterinary medicine, increased attention to transfusion medicine and concomitant success has been achieved with access to more reliable blood group determination techniques [Feldman, 1999; Giger, 2000; Giger et al., 2005; Stieger et al., 2005]. Similarly, “over-the-counter” blood group technology has

been available for human medicine with cross-matching largely automated in hospital or commercial laboratories. This study was initiated to address the lack of simple, reliable, practical blood group determination that was considered to have negative effect on available great ape patient care in zoos. Previous studies have described blood groups for laboratory-housed common chimpanzees in sizeable numbers (~500 individuals), but did not use modern techniques for patient-side testing or have populations managed for genetic diversity as occurs in zoos. Furthermore, studies of blood groups of the other great ape species [bonobo (<10), gorilla (<30)] were documented to the literature in limited numbers or were unspecified to species [orangutans (~100)], so that descriptive blood types within populations of these animals was not possible previously [Wiener et al., 1963, 1976; Wiener and Moor-Jankowski, 1972; Socha and Moor-Jankowski, 1978; Socha et al., 1984]. If validated by molecular techniques and applied to both zoo and *in situ* populations, it was hypothesized that patient-side human blood group determination methods could be used to categorize blood groups for each great ape species. This information would then be useful to develop donor databases for each species and reduce the search time for critical patient care. Similarly, ongoing breeding management could incorporate this information to minimize maternal–fetal blood interactions.

METHODS

The study protocol was approved by Lincoln Park Zoo's Research Committee and individual participating institutions as dictated by their research guidelines. Endorsement of the project was provided by the Species Survival Plan[®] (SSP) for each of the great ape species: bonobo (*Pan paniscus*), common chimpanzee (*Pan troglodytes*), gorilla (*Gorilla gorilla*), and orangutan (*Pongo pygmaeus*).

Animals

Zoo populations

SSP and European Endangered Species Program (EEP) participants were solicited through their veterinary staffs to opportunistically perform human monoclonal antibody blood group determination during routinely scheduled sedations for their collections. Sufficient individual identification was requested to positively identify the individual throughout and subsequent to the study, and included history on source (zoo-born vs. wild-origin), age, gender, and studbook number assigned within the managed population (Table 1). Over the study period

TABLE 1. Composition of Species Survival Program[®] (SSP) Great Ape Populations (*N* = 938) at the Time of Study Initiation (May 2006)^a

Bonobo	72
Common chimpanzee	334
Western lowland gorilla	339
Orangutan	225
Bornean orangutan	99
Sumatran orangutan	88
Hybrid orangutan	38

^aNumbers provided by SSP coordinators for each species at time of project endorsement.

(May 2006–February 2010), blood groups were recorded from bonobo ($n = 51$; 69% of SSP population), common chimpanzee ($n = 135$; 33% of SSP population), Western lowland gorilla (*Gorilla gorilla gorilla*) ($n = 154$; 43% of SSP population), and Bornean (*Pongo pygmaeus pygmaeus*) ($n = 35$; 26% of SSP population), Sumatran (*Pongo pygmaeus abelli*) ($n = 33$; 38% of SSP population), and hybrid (*P. pygmaeus* sp.) ($n = 17$; 39% of SSP population) orangutans.

In situ populations

Sanctuaries within the home ranges of each great apes species were solicited through their veterinary staffs to opportunistically perform human monoclonal antibody blood group determination during routinely scheduled sedations for their collections. Sufficient individual identification was requested to positively identify the individual throughout and subsequent to the study, and included history on location of acquisition, age, gender, and name or number assigned within the managed population. Over the study period (May 2006–February 2010), blood groups were recorded from bonobo ($n = 20$), common chimpanzee ($n = 98$), Eastern lowland gorilla (*Gorilla berengei grauri*) ($n = 4$), mountain gorilla (*Gorilla berengi berengi*) ($n = 1$), and Bornean ($n = 134$) orangutan.

Blood Group Determination

Participating institutions were provided with monoclonal antibody testing materials. Whole blood (<0.25 ml) was collected in EDTA ($n = 671$) or heparin ($n = 7$) anticoagulant according to the veterinarians' direction. Patented technology of a card impregnated with human monoclonal antibodies for blood groups A and B and Rh-factor (ELDONCARD 2511 and ELDONCARD 2521, identical card technology but different packaging; Eldon Biologicals A/S, Gentofte, Denmark) was utilized for this study. According to manufacturer's written directions, hydration of the monoclonal antibodies was performed with distilled water before application of a small volume (~ 0.02 ml) of anti-coagulated whole blood to each of the three reagent sites and single control on the card [www.eldonbiologicals.com Accessed: February 2006]. These instructions were provided to each institution as a summary from the package insert; additionally, when requested or if the card completion was determined unsuccessful, the instructions were supplemented as a video demonstration. Institutions in nonprimary English speaking institutions were provided translations of the summarized written instructions as appropriate to their location; versions were made in Danish, Dutch, French, German, Italian, and Spanish by zoo veterinarians primarily fluent in the language (Fig. 1).

Test results were compared with the manufacturer-provided chart to determine the individual's blood group; if an animal was group A or B, visible agglutination or cell clumping was observed within the area labeled for anti-A or anti-B antibodies, respectively; group AB would produce visual agglutination in both areas and group O was determined by a lack of reaction to either anti-A or anti-B antibodies. Similarly, Rh-factor was positive if visible agglutination was present at the site labeled D, indicating in humans the presence of Rh-positive RBCs. Control areas were not expected to agglutinate; if agglutination occurred, the card was considered invalid. The attending veterinarian marked the card with the determined blood group and confirmed the appropriate individual identification. Upon completion of the card, the whole blood applied to the card was permitted to dry entirely and the

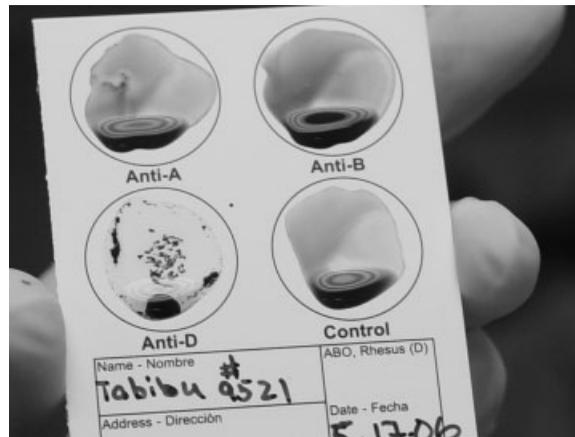


Fig. 1. This ELDONCARD was completed for an adult gorilla (*Gorilla gorilla*). It is interpreted as blood group O as no agglutination was present at either the anti-A or anti-B sites. Rh-factor is interpreted as positive due to visible agglutination present at the anti-D site.

test area was sealed with the provided self-adhesive clear laminate to create a permanent record. Project coordinators (Gamble, Moyses) received these completed cards by mail for animals housed within the United States or by digital image submitted electronically for animals housed outside the United States. Once received by coordinators, appropriate completion of the card and result interpretation was confirmed independently by each coordinator before results were entered into a database for each species.

Blood Group Testing Reliability and Validity

Card

Reliability of card technology performance in each of the great ape species was assessed by side-by-side card repetition with an identical blood sample. This procedure was performed in bonobo ($n = 22$), common chimpanzee ($n = 8$), Western lowland gorilla ($n = 29$), and Bornean ($n = 5$), Sumatran ($n = 7$), and hybrid ($n = 3$) orangutan.

Genotyping

Results from monoclonal antibody testing were validated by direct sequencing of DNA from selected animals to determine a genotype. Individuals that were selected for these studies were limited to US SSP populations, owing to international shipping requirements impacting transfer of whole blood from great apes as both nonhuman primates and endangered species. This evaluation was not successful for all the samples obtained for this procedure ($n = 118$) owing to low DNA yield, but was conclusive for genotypes in 77 animals: bonobo ($n = 10$), common chimpanzee ($n = 35$), Western lowland gorilla ($n = 26$), and Bornean ($n = 1$), Sumatran ($n = 2$), and hybrid ($n = 3$) orangutan. Detailed description and analysis of the DNA sequencing data will be presented elsewhere [Thompson et al., in preparation].

Initially, anticoagulated whole blood (~ 0.05 ml) in cell lysis buffer [0.1 M Tris-HCl (pH 8.0), 0.1 M EDTA, 0.1 sodium chloride, and 2% (w/v) sodium dodecyl sulfate] ($n = 49$) was requested for this procedure. However, DNA harvest from this

type of sample had insufficient quantity in 55% of the samples [Thompson et al., in preparation]. Therefore, whole blood in EDTA ($n = 69$) was requested subsequently. In this type of sample, DNA quality was insufficient owing to primer failure in 20% of the samples [Thompson et al., in preparation].

EDTA-anticoagulated whole blood (~2.5 ml) which had been sampled previously by monoclonal antibody technology was frozen in the collection tube and shipped on dry ice to Lincoln Park Zoo. These samples were maintained frozen at -80°C until analyses were performed in the Department of Human Genetics at the University of Chicago. Samples were thawed in a 37°C water bath at the time of processing for genotyping. From the whole blood, DNA was extracted using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, Minnesota) as per the manufacturer's directions. For blood submitted in cell lysis buffer, samples were stored at room temperature. Proteinase K was added for DNA extraction.

Following DNA extraction, the two exons in the ABO gene that determine human ABO genotype, 6 and 7, were amplified by PCR in two separate amplicons using the human DNA sequence as the reference [Thompson et al., in preparation]. Sequencing reactions were performed using a Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California). Chromatograms were aligned and analyzed using PolyPhred [Nickerson et al., 1997] with the chimpanzee ABO gene as a reference sequence [<http://www.ncbi.nlm.nih.gov/gene> Accessed: April 2007]. It was not possible to address the reliability of the monoclonal antibody technique for Rh-factor because the Rh-factor gene(s) in great apes were not successfully amplified in the common chimpanzees assessed; so, were not pursued in other species in this study.

RESULTS

Although human ABO blood groups are not identical at the DNA sequence level between or among great ape species, these label designations are maintained to be consistent with earlier reports [Socha and Moor-Jankowski, 1978].

Blood Groups

Overall, 680 animals representing all great ape species were evaluated by Eldocard with only two samples discarded for agglutinated control sites. All bonobos ($n = 71$) of both zoo-born and wild-origin presented blood group A by monoclonal antibody testing (Table 2). The common chimpanzee samples ($n = 233$) were predominantly blood group A, although a minority of the zoo-housed [SSP ($n = 3$), EEP ($n = 4$) (5.2%)] and *in situ* [$n = 29$] (29.6%)] animals were blood group O (Table 3). In SSP and EEP populations, wild-origin animals were more likely to have blood group O (11.4%) when compared with their overall group. Gorillas in SSP ($n = 147$) and EEP ($n = 7$) populations included in this study represented a single species (Western lowland gorilla) and were generally zoo-born, although 14.3% were wild-origin. The *in situ* gorilla samples had very limited representation of two species: Eastern lowland gorilla ($n = 4$) and mountain gorilla ($n = 1$). In all gorilla samples, the assigned blood group was O, except for two zoo-born SSP animals that were group A (Table 4). The SSP and EEP included Bornean, Sumatran, and Bornean–Sumatran hybrid orangutans, whereas the *in situ* population was comprised exclusively of Bornean orangutans (Table 5). Blood groups A, B, and AB were identified in all

TABLE 2. Blood Groups of Bonobo (*Pan paniscus*) ($n = 71$) by Monoclonal Antibody Analysis

Population ^a	Blood group			
	A	B	AB	O
SSP	50 ^b	0	0	0
EEP	1 ^b	0	0	0
<i>in situ</i>	20 ^c	0	0	0
Total ^d	71	0	0	0

^aSSP, Species Survival Program[®]; EEP, European Endangered Species Program; Lola ya Bonobo (*in situ*).

^bRh-factor was positive in all animals, except two zoo-born animals (SSP $n = 1$, EEP $n = 1$).

^cRh-factor was positive ($n = 9$) and negative ($n = 11$) for this group.

^dZoo-born animals were found in SSP ($n = 43$) and EEP ($n = 1$) populations. Wild-origin animals were found in SSP (7) and *in situ* ($n = 20$) populations.

TABLE 3. Blood Groups for Chimpanzee (*Pan troglodytes*) ($n = 233$) by Monoclonal Antibody Analysis

Population ^a	Blood group			
	A	B	AB	O
SSP	106 ^b	0	0	3 ^c
EEP	22 ^d	0	0	4 ^e
<i>in situ</i>	69 ^f	0	0	29 ^g
Total ^h	197	0	0	36

^aSSP, Species Survival Program[®]; EEP, European Endangered Species Program; [Centre de Conservation pour Chimpanzees, Chimfunshi Wildlife Orphanage, Limbe Wildlife Centre, Ngamba Island Chimpanzee Sanctuary, Sanaga-Yong Chimpanzee Rescue Center, Tchimpounga Chimpanzee Rescue Centre] (*in situ*).

^bRh-factor was positive ($n = 56$) and negative ($n = 23$) in zoo-born animals. Rh-factor was positive ($n = 26$) and negative ($n = 1$) in wild-origin animals.

^cRh-factor was positive in zoo-born ($n = 1$) and wild-origin ($n = 2$) animals.

^dRh-factor was positive in zoo-born ($n = 18$) and wild-origin ($n = 4$) animals.

^eRh-factor was positive in zoo-born ($n = 2$) and wild-origin ($n = 2$) animals.

^fRh-factor was positive ($n = 53$) and negative ($n = 16$) for wild-origin animals.

^gRh-factor was positive in wild-origin ($n = 23$) and sanctuary-born ($n = 1$) animals. Rh-factor was negative in wild-origin ($n = 5$) animals.

^hZoo-born animals were found in SSP ($n = 80$) and EEP ($n = 20$) populations and one sanctuary-born animal occurred in the *in situ* population. Wild-origin animals were found in SSP ($n = 29$), EEP ($n = 6$), and *in situ* ($n = 97$) populations.

Bornean orangutan populations and blood group O also was identified in one EEP individual; blood groups A and AB were identified in Sumatran orangutans; blood groups A, AB, and O were identified in hybrid orangutans.

Rh-Factor

Nearly all bonobo in SSP and EEP populations were Rh-positive, whereas 55% of the *in situ* individuals were Rh-negative (Table 2). Similarly, common chimpanzees of blood group A were predominantly Rh-positive, although some

TABLE 4. Blood Groups of Gorilla (*Gorilla* spp.) ($n = 159$) by Monoclonal Antibody Analysis

Population ^a	Blood group			
	A	B	AB	O
SSP ^b	2 ^c	0	0	145 ^d
EEP ^b	0	0	0	7 ^e
<i>in situ</i> ^f	0	0	0	5 ^g
Total ^h	2	0	0	157

^aSSP, Species Survival Program[®]; EEP, European Endangered Species Program; Mountain Gorilla Veterinary Program (*in situ*).

^bWestern lowland gorilla (*Gorilla gorilla gorilla*).

^cRh-factor was positive ($n = 1$) and negative ($n = 1$) in these zoo-born animals.

^dRh-factor was positive in all wild-origin animals ($n = 20$) and majority of zoo-born animals ($n = 116$). Rh-factor was negative in a limited number of zoo-born animals ($n = 9$).

^eRh-factor was positive in zoo-born ($n = 5$) and wild-origin ($n = 2$) animals.

^fEastern lowland gorilla (*Gorilla berengei grauri*) ($n = 4$), mountain gorilla (*Gorilla berengei berengei*) ($n = 1$).

^gRh-factor was positive in all individuals.

^hZoo-born animals were found in SSP ($n = 127$) and EEP ($n = 5$) populations. Wild-origin animals were found in SSP ($n = 20$), EEP ($n = 2$), and *in situ* ($n = 5$) populations.

animals in both SSP and EEP (19%) and *in situ* (23%) populations (Table 3) were Rh-negative; in blood group O chimpanzees, Rh-positive status predominated. Aside from a limited number of zoo-born individuals ($n = 6\%$) in SSP and EEP populations, gorilla were Rh-positive (Table 4). In orangutan species, animals were Rh-positive, except for 53% of the Sumatran orangutan of blood group A and all hybrid orangutan of blood group O (Table 5).

Test Reliability

Card

Identical results were obtained from the same blood sample on duplicated cards in all animals.

Genotyping—blood groups

Molecular technology confirmed at the genetic level that blood group A in bonobos, chimpanzees, and orangutans were identical to humans at the four sites within the ABO gene that determines human blood group A (Table 6); however, this determination does not indicate compatible blood group A between species because incompatibility could still result from other differences in the DNA sequence that were observed. In fact, slight genetic diversity was present in some common chimpanzee blood group A (Table 6). No chimpanzees with blood group O were available for molecular genotyping. In Western lowland gorilla, only samples which were group O by Eldoncard were available for molecular genotyping. This blood group was found not comparable to any human blood group. Although it presented notable intraspecific diversity of genotype, some of the patterns were similar to the genotype-based blood groups of orangutans (Table 6). In addition to blood group A in orangutans, blood group AB ($n = 3$) was evaluated by genotyping

TABLE 5. Blood Groups of Orangutan Species (*Pongo pygmaeus* sp.) ($n = 219$) by Monoclonal Antibody Analysis

Population ^a	Blood group			
	A	B	AB	O
<i>Bornean orangutan</i>				
SSP	12 ^b	3 ^b	11 ^c	0
EEP	2 ^b	1 ^b	5 ^d	1 ^b
<i>in situ</i>	37 ^e	28 ^b	69 ^f	0
Total ^g	51	32	85	1
<i>Sumatran orangutan</i>				
SSP	30 ^h	0	3 ⁱ	0
EEP	0	0	0	0
<i>in situ</i>	0	0	0	0
Total ^g	30	0	3	0
<i>Hybrid organutan</i>				
SSP	6 ^b	0	7 ^b	2 ^j
EEP	2 ^k	0	0	0
<i>in situ</i>	0	0	0	0
Total ^g	8	0	7	2
Total all species	89	32	95	3

^aSSP, Species Survival Program[®]; EEP, European Endangered Species Program; Nyaru Menen Orangutan Rehabilitation Center (*in situ*).

^bRh-factor was positive in all animals. With this footnote, all SSP animals were all zoo-born; all EEP animals were zoo-born except for one wild-origin animal; and all *in situ* animals were wild-origin.

^cRh-factor was positive in zoo-born ($n = 8$) and wild-origin animals ($n = 2$). Rh-factor was negative in zoo-born animals ($n = 1$).

^dRh-factor was positive ($n = 4$) in zoo-born animals and negative ($n = 1$) in a wild-origin animal.

^eRh-factor was positive in all but one of these wild-origin animals.

^fRh-factor was positive in the majority ($n = 66$) but negative ($n = 3$) in a limited number of these wild-origin animals.

^gZoo-born animals were found in SSP [Bornean ($n = 24$), Sumatran ($n = 30$), hybrid ($n = 15$)] and EEP [Bornean ($n = 8$), hybrid ($n = 2$)] populations. Wild-origin animals were found in SSP [Bornean ($n = 2$), Sumatran ($n = 3$)], EEP [Bornean ($n = 1$)], and *in situ* ($n = 134$) populations.

^hRh-factor was positive in zoo-born ($n = 12$) and wild-origin animals ($n = 2$). Rh-factor was negative for zoo-born ($n = 15$) and wild-origin animals ($n = 1$).

ⁱRh-factor was positive ($n = 1$) and negative ($n = 2$) in these zoo-born animals.

^jRh-factor was negative in all animals.

^kRh-factor was negative in one and positive in one zoo-born animal.

and these animals each presented a unique sequence that differed from one other (Table 6). Although each animal had sequences not observed in humans, they did have limited similarity to gorilla sequences of this study [Thompson et al., in preparation].

Rh-factor

The Rh-gene could not be amplified in the DNA samples from the common chimpanzee samples attempted and other species were not evaluated. Therefore, the validity of the monoclonal antibody testing for this profile could not be determined in this study.

TABLE 6. Genotype Blood Group Verification Against Monoclonal Antibody Blood Group in Great Apes [bonobo ($n = 10$), Common Chimpanzee ($n = 35$), Western Lowland Gorilla ($n = 26$), Orangutan ($n = 6$)] [Ober et al., in preparation]

	Monoclonal antibody blood group	Genotype
Bonobo	A	A/A
Common chimpanzee	A ($n = 31$)	A/A
	A ($n = 4$)	A/non-A, non-B ^a
Western lowland gorilla	O ($n = 8$)	Non-A, non-B
	O ($n = 15$)	Non-A, non-B
	O ($n = 3$)	Non-A, non-B
Orangutan	A ^b ($n = 3$)	A/A
	AB ^c ($n = 1$)	Non-A, non-B
	AB ^d ($n = 1$)	Non-A, non-B
	AB ^c ($n = 1$)	A/non-A, non-B

^aSimilar deletion in exon 7 [Kermerrac, 1999].

^bSumatran orangutan ($n = 2$), hybrid orangutan ($n = 1$).

^cHybrid orangutan ($n = 1$).

^dBornean orangutan ($n = 1$).

DISCUSSION

Access to great ape whole blood for blood group determination was variable, and widely spaced in time, during historic comparative human blood group literature [Socha et al., 1984]. Notably, blood group studies in bonobo were not determined until 1970 and remain as limited studies in this species [Moor-Jankowski et al., 1972, 1975; Weiner and Moor-Jankowski, 1972; Socha and Moor-Jankowski, 1978; Socha et al., 1984]. The findings of this study of exclusive blood group A for bonobo was in agreement with that reported in these citations.

In contrast, common chimpanzees were available routinely from laboratory situations and so were assessed in large numbers (~500 individuals) from as early as 1911 [Socha and Moor-Jankowski, 1978; Socha, 1981; Socha et al., 1984] and smaller *in situ* numbers ($n = 40$) in the 1960s [Eyquem et al., 1962]. Predominantly, blood group A was reported in common chimpanzees throughout this literature, with group O present comparably (2.5–12%) to that in SSP and EEP collections of this study (5.2%) [Weiner and Gordon, 1960; Wiener et al., 1963; Eyquem et al., 1962; Moor-Jankowski and Wiener, 1964; Socha and Moor-Jankowski, 1978]. However, the strictly wild-origin animals of the *in situ* population of this study demonstrated a larger presence (29.6%) of blood group O when compared with the overall group. When only wild-origin individuals are considered from the SSP and EEP population, blood group O (11.4%) was more common in the wild-origin SSP and EEP animals as compared with the overall group housed in zoos (5.2%). In review of the literature, studies documented earlier than 1940, when larger numbers of the captive chimpanzee population would have been of wild-origin, a larger presence of blood group O (12–20%) was reported as compared with those studies conducted after 1960 (9.4–14.9%) [Weiner and Gordon, 1960; Weiner and Moor-Jankowski, 1972; Socha and Moor-Jankowski, 1978].

Given that laboratory populations are considered closed and conservatively maintained for purposes of study design, a trend toward reduced genetic diversity is not surprising [Weiner and Gordon, 1960; Socha, 1981]. Cooperative management

programs, such as the SSP, are managed to slow the loss of genetic diversity from populations that are increasingly captive born. Even so, it was considered probable that extinguishing blood group O from the SSP chimpanzee population will occur [Penedo, 1993]. Interestingly, although the number of chimpanzees from the EEP ($n = 26$) was much smaller than that evaluated from the SSP ($n = 109$) in this study, it had a larger presence of blood group O, 18 vs. 2.8%. This finding may be artificial from these fewer numbers assessed against the US population. However, it may be real owing to different management of zoo great ape populations of Europe and North America.

As with bonobo, relatively few gorillas have been studied previously. In those studies, gorillas ($n = 23$) were not assigned to species, so they could be either Western or Eastern lowland subspecies [Wiener et al., 1976; Socha and Moor-Jankowski, 1978]. Regardless, gorilla blood groups were uniformly noted as nonreactive to anti-A or anti-B antibodies; so, were assumed to be group O [Weiner and Gordon, 1960; Socha et al., 1972; Socha and Moor-Jankowski, 1978]. On the other hand, body fluids (saliva and urine) were weakly, but uniformly, assigned to group B [Weiner and Gordon, 1960; Wiener et al., 1963; Moor-Jankowski and Wiener, 1964; Socha and Moor-Jankowski, 1978; Socha et al., 1984; Crouse and Vincek, 1995]. In this study, gorillas again were considered nonreactive or blood group O by monoclonal antibody technology. In comparison to lowland gorillas, access to clinical samples from mountain gorillas has never been widely available for blood group determination. Initially, no reported differences were noted between lowland and mountain gorilla ($n = 2$) blood group assessment [Socha et al., 1973]. However, one researcher—and a coauthor in the lowland gorilla study—consistently remarked that mountain gorillas were similarly nonreactive as the lowland species when blood was utilized, but were group A on secretor status [Weiner and Gordon, 1960; Wiener et al., 1963]. Sample sizes were quite small ($n = 4$), and in a later report it was commented that no differences existed in their interpretation between the lowland and mountain gorilla species [Wiener et al., 1976].

For this study, nearly uniform blood group O for the gorilla population is not a misinterpretation from this previous documentation, as no reactivity had been previously assessed in the literature from whole blood. Additionally, in humans, blood group O is produced by minor genotypic change on chromosome 9 which truncates the H antigen on the RBCs [Kermarrec et al., 1999]. However, this same effect could be produced by any number of comparable genetic changes that abrogates the conversion of the H antigen to type A or B [Kermarrec et al., 1999; Seltsam et al., 2003; Yazer, 2005]. In gorillas, no reaction occurred with the human anti-A and anti-B monoclonal antibodies and would be assigned blood group O by this method. However, by genotyping in this study, gorillas had a novel sequence that differed from both human A and B groups and would not necessarily abrogate the gene function, as occurs in human group O blood. This study demonstrates that the species has a diverse blood group system that differs from the human ABO blood groups [Thompson et al., in preparation].

As recently as 1999 [Kermarrec et al., 1999], literature suggested that no ape species displayed all four human blood group phenotypes: A, B, AB, and O [Moor-Jankowski and Weiner, 1964; Wiener et al., 1963; Socha and Moor-Jankowski, 1978]. The numbers of orangutans in this study ($n = 219$) is not only twice that of all previous studies combined, but also delineates between species and hybrid status

[Socha and Moor-Jankowski, 1978; Socha et al., 1984; Wiener et al., 1963]. Previous studies reported blood groups of A, B, and AB in orangutans [Weiner and Gordon, 1960; Socha and Moor-Jankowski, 1978; Socha et al., 1984], with the exception of one individual (species unspecified) that was blood group O [Wiener et al., 1963]. In this study, all four human blood phenotypes were present by monoclonal antibody technology. Although this diversity also was present specifically in Bornean orangutans ($n = 169$), they represented the larger proportion of the samples evaluated; Sumatran orangutans were identified with blood groups A and AB and groups A, AB, and O were present in hybrids (Table 5).

The complete range of blood group diversity was documented in only Bornean and not in Sumatran orangutans. Therefore, the blood group distribution in the zoo-born orangutan hybrids could be simply a result of interspecies breeding. None of the individual hybrids ($n = 15$) included in this study were younger than 23 years of age and born, therefore, before more effective contraception and population management for this species had been made available and hybridization was discouraged. Hybridization parentage by sire and dam may impact the overall hybrid distribution of blood groups as has been noted in domestic animal hybrids [Eyquem et al., 1962], but it was not possible to assess this effect from this study data.

Human and great ape blood group A as determined by monoclonal antibody technology and genotyping were identical at four sites within the ABO gene that determines human blood group A, but this does not mean they are interchangeable interspecifically (Table 6). However, this study confirmation does support use of monoclonal antibody technology (Eldoncard) for patient-side typing in these animals. Despite this confirmation, it has been documented in the literature that a single transfusion from a human to a common chimpanzee could be tolerated, although the converse was not true [Wiener and Moor-Jankowski, 1972], as common chimpanzees have been determined innately to not have agglutinating antibodies to human blood types while humans do possess such agglutinins. In blood group O chimpanzees as with gorilla, although the blood was nonreactive with human anti-A and anti-B and, therefore, group O, the gene controlling ABO group determination may not create a nonreactive protein. In fact, as no common chimpanzee blood group O individuals were available for genotyping, it is unclear if they themselves may be reactive with the species. Furthermore, in the case of the gorilla, genotyping determined that human blood groups are not detectable or comparable (Table 6) by human monoclonal antibody analysis. This finding represents a failure of the monoclonal antibody technology for this species. Orangutans with blood groups A and AB were available for genotyping; although A was quite consistent at the four sites evaluated, AB presented considerable diversity. As individuals with blood group B or O were not available for genotyping, similar comments cannot be concluded. However, it is suggested that monoclonal antibody technology can be utilized cautiously in this species to assign blood groups.

In addition to blood group-associated antibodies, other hemoactive proteins, such as Rh-factor, exist [Feldman, 1999; Knottenbelt, 2002]. This latter protein has become well described in human medicine literature as the predominant source for maternal-fetal blood interactions [Weiner et al., 1977; Kendig, 2007]. Primate placental attachments are hemochorial and so permit close circulatory contact between the mother and fetus. When an Rh-negative mother carries a fetus with Rh-positive blood inherited from the father, close association of maternal and fetal

blood presents exposure of the Rh-antigen to the mother with subsequent development of antibodies to this factor [Kendig, 2007]. Exposure of the Rh-positive fetus to these maternal antibodies presents essentially a mistransfusion where RBC destruction (neonatal isoerythrolysis) and its resultant systemic effects occur, resulting in *in utero* death, stillbirth, or delivery of weak neonates [Weiner et al., 1977; Kendig, 2007]. Sensitization from prior pregnancies is the most common situation associated with this maternal–fetal incompatibility in humans. However, antepartum hemorrhage, spontaneous abortions, ectopic pregnancies, abdominal trauma during pregnancy, including clinical procedures of amniocentesis, chorionic villous sampling, or fetal surgery, blood mistransfusions, and twinning, can similarly produce alloimmunization situations [Weiner et al., 1977; Kendig, 2007].

Rh-factor is clearly not the only circulating antigen and others would have potential to produce similar reactions in great ape gestations; in fact, this situation has been documented in common chimpanzees and orangutans [Weiner et al., 1977; Socha and van Foreest, 1981]. Additionally, this issue could be associated with differing parent ABO blood groups [Weiner and Gordon, 1960; Weiner et al., 1977]. Prevention of these responses can be accomplished by foreknowledge of blood groups of the parents which permits appropriate *in utero* fetal support or immediate neonatal intervention [Weiner et al., 1977; Socha and van Foreest, 1981; Socha et al., 1984; Kendig, 2007]. Therefore, parental analysis for breeding recommendations and evaluation of subsequent pairings will need cross-matching analysis as occurs with blood transfusion [Weiner et al., 1977; Giger, 2000; Knottenbelt, 2002].

Blood group determination reveals potential donors from the population at large [Socha et al., 1984]. For all great ape species, except gorilla, the individuals' blood groups have been cataloged by species as a database to provide quick reference for those clinicians faced with clinical situations where transfusion is planned or has occurred. However, for all great apes, as in humans, cross-matching will be necessary to confirm donor selections and, when properly completed, minimize mistransfusion [Socha et al., 1984]. This technique assesses for the presence of conflicting antibodies by separating the donor and recipient blood into its cellular (RBCs) and acellular (serum) components [Feldman, 1999; Knottenbelt, 2002]. *In vitro* exposure of donor RBCs to recipient serum (major cross-match) and donor serum to recipient RBCs (minor cross-match) is completed and evaluated for agglutination both grossly and microscopically. Although not providing a specific blood group, this determination can prevent clearly evident transfusion mismatch and is quick, easy, inexpensive, and requires little blood to complete [Feldman, 1999; Knottenbelt, 2002]. The addition of this information to the species-specific pedigree database will go toward increasing successful blood transfusions with reduced donor risk in zoological collections [Socha, 1981].

Blood transfusion complications typically arise as urgent clinical situations and include shock and hemodynamic collapse resulting from RBC destruction. Although cross-matching can assist the immediate problem, it does require whole blood collection from both donor and recipient which generally requires sedation, even in zoological collections. Knowledge of blood types and cross-matching status in advance of these problems would be ideal. Because all great ape species are secretors (i.e., blood group antigens can be detected in other body fluids, such as saliva), this advance knowledge may some day be possible if appropriate testing can be developed [Wiener et al., 1963; Socha et al., 1984; Moor-Jankowski and Wiener, 1964]. Finally,

this study has made clear that clinical transfusion interests aside, determination of great ape groups have other applications that warrant continued investigation of the great ape blood group genotypes.

CONCLUSIONS AND CLINICAL IMPLICATIONS

Bonobo are exclusively blood group A by monoclonal antibody technology. These animals as well as blood group A common chimpanzees and orangutans have the same genotype at the four sites that determine the human blood group A.

By monoclonal antibody technology, common chimpanzees are predominantly blood group A, although limited portions of the SSP and EEP populations had blood group O. Wild-origin individuals were more likely to be group O than zoo-born animals. The latter blood group may be a true nonreactive O without expression on RBCs or may represent a novel, chimpanzee-specific allele.

Nearly all gorillas did not react to the antibodies of the monoclonal antibody technology which was consistent with blood group O phenotype. However, genetic studies revealed diverse blood groups that were not similar to human blood group A, B, or O.

Orangutans in the genus and Bornean species have all four human blood group phenotypes—A, B, AB, and O—identified by molecular antibody technology which suggested this species as the first nonhuman primate to present all four human blood group phenotypes, although only blood group A was similar at the genotype level to the human blood group A.

Bonobo and common chimpanzees had uniform or predominant blood groups throughout zoo populations. Although these blood transfusions can be conducted without much risk of adverse reactions, when such procedures involve wild-origin or founder common chimpanzees in SSP and EEP populations, higher frequency of blood group O may complicate identification of successful donors or present issues with maternal–fetal mismatch. In any transfusion situation, cross-matching of individuals is recommended strongly before transfusion and essential when wild-origin animals are involved.

Because gorillas do not have human A, B, or O blood groups, even single blood transfusions have risk of adverse reaction. Cross-matching of individuals is essential in this species before transfusion.

Orangutan blood groups were diverse, regardless of population. Because hybrids are present in zoo populations, ongoing blood group assessment is recommended to target appropriate transfusion candidates ahead of actual need, and cross-matching of individuals is essential before transfusion even in those individuals within a consistent blood group.

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