age of ~37 million years for this meteorite, a rather long space exposure considering that a majority of H-chondrites have nominal cosmic-ray space exposure age of ~8 million years. The measured activities of the different cosmic-ray produced radionuclides (^{7}Be, ^{56,58}Co, ^{46}Sc, ^{57}Co, ^{54}Mn, ^{22}Na, ^{60}Co and ^{26}Al) with half lives varying from 53.3 days to 0.7 million years are at saturation level, which is expected because of the very long space exposure duration of the meteorites. Combining noble gas data and observed records of galactic cosmic-ray produced nuclear tracks in the six spot samples analysed by us, it is possible to infer the degree of atmospheric ablation suffered by the Kasauli meteorite during its atmospheric transit. Our preliminary data suggest this to be ~78%, which is within the typical range (70–85%) inferred for most single-meteorite falls.

Note added in the proof: Following the submission of the paper, two reports appeared describing the Orissa fall (Mohanty, M. *et al.*, *Curr. Sci.*, 2004, **87**, 428–429) and chemical composition of a fragment of this fall that confirms its extra-terrestrial origin (Nair, A. C. C. *et al.*, *Curr. Sci.*, 2004, **87**, 654–657). In these reports various fragments of this multiple fall have been given informal names such as Subarnapur, West Suniti, East Suniti and Jagannath. As noted in the present paper, the name approved for this multiple fall by the Meteorite Nomenclature Committee of the International Meteoritical Society is Kendrapara (ref. 5). Thus all fragments of this multiple fall, including those reported in the above two papers, should be referred to as fragments of Kendrapara meteorite.

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Cloning of canine parvovirus VP2 gene and its use as DNA vaccine in dogs

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The VP2 gene of canine parvovirus (CPV) was amplified by polymerase chain reaction and cloned in pTargeT mammalian expression vector. The recombinant plasmid containing VP2 gene in right orientation was selected on the basis of restriction enzyme analysis and further confirmed by sequencing. The recombinant plasmid pTargeT.cpvp2 was used to transfect CRFK cells and found to express VP2 protein as detected by immunoperoxidase test. It was used as DNA vaccine in dog by injecting as 100 μg DNA with and without adjuvant, and keeping the vector alone and healthy control groups. The dogs injected with recombinant plasmid alone or with adjuvant ISA50 (10% v/v) were protected fully from the disease, whereas those injected with vector alone and healthy control dogs died/suffered from the disease. The serum neutralizing antibody titre in vaccinated dogs was 1 : 256 and 1 : 512, while vector alone and healthy control group dogs did not show any serum neutralization antibody response. The present work has shown that the recombinant plasmid could be used as DNA vaccine against canine parvovirus infection.

CANINE parvovirus (CPV) is a member of the parvovirus genus which was first recognized[1,2] in 1978; it is a small non-enveloped, single-stranded DNA virus[3]. It replicates autonomously, causes severe enteritis in dogs of all ages and myocarditis in pups less then 12 weeks of age. The organization of CPV capsid is similar to that of other autonomous parvoviruses, in that it contains two largely overlapping proteins, VP1 (82 K) and VP2 (65 K)[4–6]. There is a third protein, VP3 (63 K), which is produced by proteolytic processing of VP2. The 22 nm parvovirus capsid comprises[7,8] about ten copies of VP1 and 60 to 70 copies of VP2. Epitope mapping experiment showed that all of the epitope-neutralizing antibodies are within VP2 and a T cell epitope was also found in the VP1-specific region[9]. CPV infection is quite common and the disease is usually controlled using a live or inactivated vaccine, although maternally derived antibodies may inhibit the replication of live attenuated vaccines in puppies having temporary immunity[10,11].

DNA vaccines induce immunity by transfection of host cells with a non-replicating plasmid DNA that expresses immunogenic proteins or antigenic epitopes. This novel approach has the potential to revolutionize vaccination as a method for infectious disease control in animals and humans. DNA vaccines induce both humoral and cellular

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immunity, do not replicate, are incapable of infecting the host, are not difficult to produce, allow presentation of viral antigens in the native form and are stable at room temperature for long periods. These have improved cross-strain protection and allow presentation of viral antigens in the native form[12]. These characteristics make DNA vaccines a favourable alternative approach to conventional and recombinant vaccines[13]. The purpose of this study was to determine whether DNA immunization could induce protective immunity in dogs against CPV infection.

A permanent line of feline kidney cells (CRFK) was used for virus production. CRFK cells were propagated in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum and antibiotics.

The infected dog faeces containing CPV confirmed by PCR was used to isolate the virus in CRFK cells and maintained in the molecular virology laboratory.

The viral DNA was isolated from 200 µl sample using QIAamp DNA Mini kit (QIAGEN) according to the manufacturer's instructions and eluted in 200 µl volume. The full-length VP2 gene was amplified using primer pair forward: 5′-GAG ACA ATC TTG CAC CAA TG-3′; reverse: 5′-GGT GCT AGT TGA TAT GTA ATA AAC-3′, self-designed based on published sequence (GenBank accession no. M19296). PCR reaction was performed using 10 µl of isolated viral DNA along with forward and reverse primers (50 pmol each), 200 µM of deoxynucleotide triphosphates, 1.5 mM $MgCl_2$ and 3U of Expand™ High fidelity DNA polymerase (Roche) in 1X reaction buffer. The cyclic conditions were initial denaturation at 94°C for 3 min and then 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and amplification at 72°C for 3 min and the final extension at 72°C for 5 min. The PCR amplified product was analysed on 1% agarose gel along with DNA molecular weight marker.

The amplified VP2 gene PCR product was characterized by nested PCR using primer set forward: 5′-TAC CAT GGT ACA GAT CCA G-3′; reverse: 5′-CCT CTA TAT CAC CAA AGT TA-3′ reported for CPV diagnosis[14]. The nested PCR reaction was set utilizing 1 µl VP2 gene PCR product as target DNA. The reaction mixture contained 50 pmol each of forward and reverse primers, 200 µM of deoxynucleotide triphosphates, 1.5 mM $MgCl_2$ and 3U of Expand™ High fidelity DNA polymerase (Roche) in 1X reaction buffer. The cyclic conditions were initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and amplification at 72°C for 30 s and final extension at 72°C for 5 min. The PCR amplified product was analysed on 1% agarose gel along with DNA molecular weight marker.

The amplified VP2 gene was gel-purified using QIAquick gel extraction kit (QIAGEN) following manufacturer's instructions. The gel-purified PCR product was 3′-A-tailed using 5 U Taq DNA polymerase (LifeTechnologies) in a reaction mixture containing 200 µM deoxyadenasine triphosphates and 2.5 mM $MgCl_2$ in 1X reaction buffer at 72°C for 30 min. This 3′-A-tailed PCR product was ligated into pTargeT eukaryotic expression vector using T4 DNA ligase (LifeTechnologies) at 16°C overnight. The ligated plasmid was transformed into *Escherichia coli* JM109 competent cells. The white colonies having recombinant pTargeT plasmids (pTargeT.cpvp2) were isolated using QIAGEN-tip 20 plasmid DNA isolation column (QIAGEN) following manufacturer's instructions. The presence of VP2 gene in pTargeT.cpvp2 plasmid was checked by restriction digestion with *Eco*RI and orientation of the VP2 gene insert was checked by *Pst*I followed by analysis of the digested products on 1% agarose gel and further confirmed by sequencing.

Sequencing of the CPV VP2 gene was done using the recombinant plasmid pTargeT.cpvp2 DNA using ABI Prism model 377 version 3.0 and the sequence was finally arrived at using DNASTAR.

The expression of the recombinant plasmid was assayed using the protocol for transient transfection of adherent cells (QIAGEN) using the effectene transfection reagent (QIAGEN) for one well of a six-well culture plate. The reagents used per well were: DNA, 0.4 µg; enhancer, 3.2 µg, final volume of DNA in DNA-condensation buffer, 100 µl; volume of effectene reagent, 10 µl; volume of medium to add to cells, 1600 µl; volume of medium to add to complexes, 600 µl. One 25 cm^2 flask containing a complete monolayer of CRFK cells was taken, the cells were dispersed using trypsin–versene solution and finally suspended in 10 ml growth medium. This was distributed into a six-well culture plate, with 1.6 ml in each well. The plate was kept in a CO_2 incubator under 5% CO_2 at 37°C for 24 h. On the day of transfection 16 µl DNA (1.6 µg) was diluted with the DNA-condensation buffer to a total volume of 400 µl in a 15 ml tube. Then 12.8 µl of enhancer was added into and mixed by vortexing. It was incubated at room temperature (25°C) for 5 min and given a short spin. Next 40 µl of effectene transfection reagent was added to the DNA-enhancer mixture and mixed by pipetting up and down five times. This was followed by incubation for 10 min at room temperature to allow transfection-complex formation. During this time, the medium was pipetted out from the six-well culture plate without disturbing the cells and washed with growth medium. Next, 1.6 ml growth medium was added in control wells and 1280 µl growth medium was added in test wells to be transfected. Then 2400 µl of growth medium containing FCS and antibiotic was added to the tube having transfection complexes. After mixing by pipetting up and down twice, 750 µl of transfection complex was immediately added dropwise into each well in the culture plate leaving the controls. The plate was gently swirled to ensure uniform distribution of the transfection-complexes. The cells were incubated for 48 h in 5% CO_2 incubator at 37°C after which the protein expressed was detected by immunoperoxidase straining.

The procedure described by Rai[15] was followed. The medium from wells of culture plate was removed and washed with PBS, pH 7.2. The cells were fixed with chilled acetone at 4°C for 10 min and then air-dried. A few drops of dog anti-

CPV antiserum were added and incubated at 37°C for 1 h in a humid chamber. Two wells served as control. The wells were washed with PBS. A few drops of the affinity isolated rabbit anti-dog IgG-peroxidase conjugate were added, and incubated at 37°C for 1 h in a humid chamber. The wells were washed three times with PBS and once with distilled water, and then were air-dried. Three drops of freshly prepared Nadi reagent were added to the preparation and allowed to react for 2 min. The preparation was rinsed briefly with PBS and treated for about 1 min with each of the following: 70%, 90% and 100% alcohol followed by a quick wash with xylene. The monolayers were observed under microscope.

The recombinant plasmid pTargeT.cpvp2 was isolated using QIAgen HiSpeed plasmid midi kit according to the manufacturer's protocol.

Two beagle dogs, one-month-old, were immunized each with 100 µg of pTargeT.cpvp2 intramuscularly at the right quadriceps muscle; two dogs with recombinant plasmid DNA along with ISA50 (10% v/v), two dogs with vector + ISA50, and the control healthy group consisted of two dogs. Blood samples were collected before and 21 days post-vaccination for all the dogs. These dogs were from unvaccinated mother and free from CPV antibodies.

All the dogs, were challenged 21days post-vaccination by inoculation with 10,000 ID50 virulent CPV in 1 ml of infective dog faeces diluted in PBS given intranasally, 0.5 ml in each nostril. Clinical manifestations of the disease were monitored for 17 days post-challenge.

Serum neutralization (SN) antibody assays were performed after heat-inactivating all serum samples at 56°C for 30 min. Assays were performed in 96-well microtitre plates by combining 0.05 ml of sera in serial twofold dilutions in cell culture medium (MEM + 10% FBS), with an equal volume of CPV suspension containing 100 TCID50 virus diluted in cell culture medium. The serum–virus mixture was then incubated for 1 h at room temperature, after which an equal volume of CRFK cells suspended in cell culture medium was added into the mixture and incubated for 3 days at 37°C with 5% CO_2. The microtitre plates were then examined under a microscope and the SN antibody titre was calculated as the reciprocal of the highest serum dilution that neutralized 50% of the virus.

The isolation of CPV DNA was successfully done and the VP2 gene could be successfully amplified using self-designed primers. The 1817-bp PCR product was cloned in pTargeT vector and the recombinant plasmid containing the VP2 gene in right orientation was selected on the basis of restriction enzyme digestion using *Pst*I, in which case the expected fragment sizes, 3.7 kb, 3.10 kb and 0.73 kb of the recombinant plasmid pTargeT.cpvp2 were observed (Figures 1–3). Sequencing of the recombinant plasmid DNA confirmed the right orientation of the VP2 gene. The gene sequence was submitted to EMBL/GenBank/DDBJ database and assigned the accession no. AJ698134 and a protein accession no. CAG 27358. The results obtained in the study are shown in Table 1. The recombinant plasmid in a dose of 100 µg given intramuscularly with and without the adjuvant gave 100% protection, although the SN antibody titre in adjuvant group was higher (1 : 512) compared with the DNA-alone group (1 : 256). Control dogs did not show any antibody response. Our results are in agreement with those of Jiang *et al.*[16], who showed that DNA vaccine could protect dogs and the vaccinated dogs did not shed virus in the faeces, while the control challenged dogs showed faecal shedding.

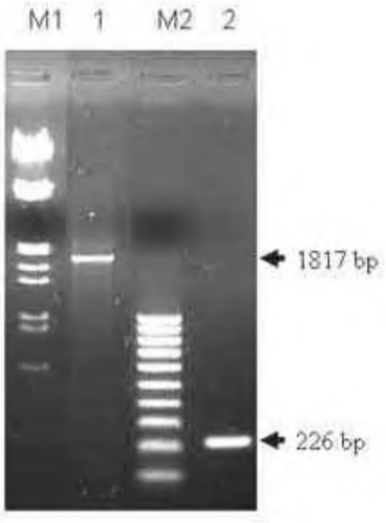

Figure 1. PCR amplification of CPV VP2 gene. M1, λDNA *Hin*dIII/*Eco*RI digested DNA marker; Lane 1, 1817 bp amplified CP VP2 gene PCR product; M2, 100 bp ladder DNA marker; lane 2, 226 bp nested PCR product.

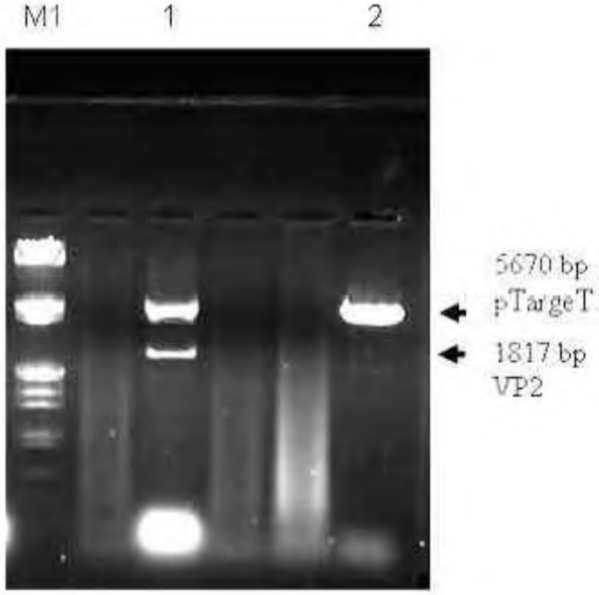

Figure 2. Cloning of CP VP2 gene into pTargeT vector. M1, λDNA *Hin*dIII/*Eco*RI digested DNA marker; lane 1, Recombinant plasmid pTargeT cpvp2 digested with *Eco*RI showing insert and vector; lane 2, pTargeT vector digested with *Eco*RI.

Table 1. CPV DNA vaccine trial in dogs*

Group	Dose (μg)	No. of dogs	No. protected	Percentage protected	Mean SN antibody titre in sera
pTargeT.cpvp2	100	2	2	100	256
pTargeT.cpvp2 + ISA50 (10% v/v)	100	2	2	100	512
pTargeT + ISA50 (10% v/v)	100	2	0	0	0
Healthy control		2	0	0	0

*All groups of dogs were challenged with 10,000 ID50 virulent CPV in the form of 220 nm membrane-filtered infected, faecal sample from CPV-infected dog, given intranasally 0.5 ml in each nostril.

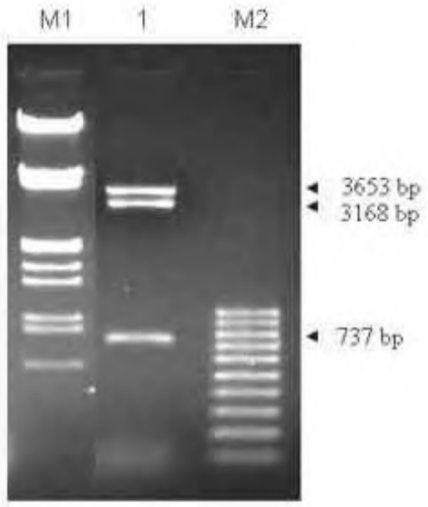

Figure 3. Confirmation of orientation of CPVP2 gene in recombinant plasmid. M1, λDNA *Hin*dIII/*Eco*RI digested DNA marker; lane 1, Recombinant plasmid pTargeT cpvp2 digested with *Pst*I for checking orientation of insert; M2, 100 bp ladder DNA marker.

Protection against CPV infection is most probably mediated through a humoral response, as shown by passive transfer of antibodies[17,18]. However, other protective mechanisms such as a cytotoxic T lymphocyte (CTL) response, cannot be excluded[12,19–23]. It is known that plasmid DNA injection into the muscle can induce antigen-specific CTL response. In this study, only humoral response was examined, which was protective; however; cellular immunity could pay a role in protection with this vaccine also.

DNA vaccines have several advantages over conventional vaccines. They are easily produced, chemically pure and stable for long periods when stored at room temperature. Since no living organisms are injected, no hazards exist for vaccine-induced disease, which is inherent in vaccines made from modified pathogens. Proteins expressed by DNA vaccine produce a continuous stream of antigens, which induce a prolonged immunostimulatory response. Since this stimulation is not interrupted by the immune response, it is possible that this type of vaccine will not be affected by passive maternal antibodies. In addition, continuous expression of CPV viral antigen that is characteristic of DNA vaccine results in better immune response than recombinant vaccine, which delivers only a single pulse of antigen and requires multiple doses to achieve protection and expresses the entire antigenic viral protein in its native form, thus providing better stimulation of the immune system. Thus, the DNA vaccine developed in the present study can be used successfully.

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In vitro growth inhibition of microbes by human placental extract

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Human placental extract that is used as a wound healer, acts as a stimulating agent for tissue repair. It has an effective inhibitory role on the growth of different microbes like bacteria, e.g. *Escherichia coli*, *Staphylococcus aureus* and fungi, e.g. *Saccharomyces cerevisiae*, *Kluyveromyces fragilis* and *Candida albicans*. It also prevents growth of clinically isolated bacteria, e.g. *E. coli* from urine and blood culture and *S. aureus* from pus. Drug-resistant strains such as *E. coli* DH5α Pet-16 Amp^R and *Pseudomonus aeruginosa* Cam^R were also significantly inhibited by the extract. The extract has both bacteriostatic and fungistatic activities. Dose-dependent response of the extract was observed. Antimicrobial activity was retained after heating but was lost after dialysis. The MIC of the extract varies between 200 and 8000 mg/l. No antimicrobial activity was observed with human serum and aqueous extract of mouse muscle serving as control. A mixture of polydeoxyribonucleotides appears to be the causative agent. Partial protection of the wound from secondary microbial infection is thus indicated.

USE of placenta as a therapeutic agent has been prevalent for a long time. It is an immunologically privileged organ and has unique pharmacological effects like enhancement of wound-healing, anti-inflammatory action, analgesic effect, etc. A variety of substances with biological and therapeutic activity present in human placenta, have been isolated and identified as hormones, proteins, glycosaminoglycans, nucleic acids, polydeoxyribonucleotides (PDRNs), etc. The composition of placental extract thus depends on the method of its preparation. Consequently, it shows different therapeutic activities[1]. In many countries, intra-muscular and topical use of the extract for burn injuries, chronic wounds and as post-surgical dressing is an age-old practice[2–6]. Under such conditions, an effective tissue-regenerative agent needs to take care of prevention of secondary bacterial or fungal infection. Our objective is to evaluate an extract developed indigenously from human placenta in terms of its functionality and active components. Scientific assessment of such an extract is necessary for its better acceptance in medical practice. Recently, presence of biologically active NADPH[7] and fibronectin type III like peptide[8] in the extract has been demonstrated. Further, different spectroscopic and chromatographic analyses have revealed high degree of consistency among different batches of the extract[9]. Here we report *in vitro* inhibition of growth of different bacterial and fungal strains by this extract.

M/s Albert David Ltd, Kolkata, India, supplied an aqueous extract of human placenta under the trade name 'Placentrex', which is manufactured under proprietary method. In short, fresh placentae stored in ice, were tested for HIV antibody and hepatitis B surface antigen. Single hot and cold aqueous extractions were done at 90 and 6°C respectively, followed by sterilization. It was filtered aseptically, benzyl alcohol was added up to 1.5% as preservative and was sterilized once again. Each millilitre of the extract was derived from 0.1 g of fresh placenta. A single batch was prepared from a pool of several placentae. Overall manufacturing procedure holding confidentiality of the proprietary terms has been described earlier[7]. Dry weight of placental extract was 10 ± 0.50 mg/ml. Benzyl alcohol at the concentration present in the extract has no prolonged effect on microbial growth[10].

Yeast extract, bacto-peptone, bacto-tryptone and agar were from HI-MEDIA Laboratories Pvt Ltd, India and D-glucose was from Qualigen, India. DNAaseI (from bovine pancreas), protease type XIII (from *Aspergillus saitoi*), dialysis tubing (cut off range < 12 kDa), including the benzoylated one (cut-off range < 2 kDa) and hydroxyapetite were from Sigma, USA.

Microbial strains collected were as follows: *Escherichia coli* (DH5α); *Saccharomyces cerevisiae* diploid strains derived from *S. cerevisiae* strains 8534-10A (MATa, leu2, ura3, his4) and 6460-8D (MATα, met 3), *Kluyveromyces fragilis* (ATCC No. 10022); ampicillin-resistant *E. coli* DH5α Pet-16 (Amp^R); chloramphenicol-resistant *Pseudomonus aeruginosa* (Cam^R) were from different laboratories of our institute. *Staphylococcus aureus* (type strain, MTCC No. 1430) and *Candida albicans* (type strain, MTCC No. 1637) were from Microbial Type Culture Collection,

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